UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

006937

OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

DEC - 9 1988

SUBJECT: Atrazine, Toxicology Chapter of the Registration Standard
Tox. Chem # 63

TO: Chuck Kent, Chief
Reregistration Branch (TS-767C)
Special Review and Reregistration Division

FROM: Marion P. Copley, D.V.M., D.A.B.T. Marion Copley 11/29/88
Acting Section Head, Review Section 2
Toxicology Branch I (IRS), HED (TS-769C)

THROUGH: Robert P. Zendzian Ph.D. 11/30/88
Toxicology Registration Standard Coordinator
Health Effects Division (TS-769C)

Albin Kocialski, Ph.D., Head
Registration Standards and Special Review Section

William Burnam, Acting Director
Health Effects Division (TS-769C) 12/2/88

Attached is the Toxicology Chapter of the Final Registration Standard and Tolerance Reassessment (FRSTR) for Atrazine. The following portions are available in Wordperfect™ format. You may obtain a copy from the reviewer.

A. Toxicology Summary
B. Toxicology Profile
C. Data Gaps
D. ADI Reassessment
E. Toxicological Issues
F. Toxicology Summary Tables

Only Data Evaluation Reports in support this FRSTR that have been completed subsequent to the initial Standard in 1983.

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TOXICOLOGY CHAPTER

OF THE FINAL REGISTRATION STANDARD

AND TOLERANCE REASSESSMENT FOR

ATRAZINE

Prepared by
Marion P. Copley, D.V.M.,D.A.B.T.
Acting Section Head, Review Section 2
Toxicology Branch 1 (IRS)
Hazard Evaluation Division

Toxicology Chapter

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A. Toxicology Summary

Atrazine (2-chloro-4-ethylamine-6-isopropylamino-S-triazine] (see Figure 1) is a chlorotriazine herbicide for season-long control in corn, sorghum and other crops. It has a low to mild acute oral (III) and dermal (III) toxicity. There are no acceptable acute inhalation studies with the technical that measure the actual exposure and particle size. Atrazine produces only slight dermal (III) irritation, moderate ocular (II) irritation and is not a dermal sensitizer.

There are no acceptable subchronic oral (dog, rat) and dermal (rat) toxicity studies. The oral oncogenicity (mice only) study presents no significant toxicologic concerns. However, there are tumors in Sprague-Dawley rats and cardiac effects in dogs.

It was determined that Atrazine has oncogenic potential in female rats resulting in mammary tumors. This data were obtained subsequent to the issuance of the United States Environmental Protection Agency (EPA) Guidance for Registration of Pesticide Products containing Atrazine, November 10, 1983 (RS-83). A core-supplementary rat chronic/ oncogenicity study (00059211), noted in RS-83, did not delineate the oncogenic potential of Atrazine due to poor survival and intercurrent infection. The new (core-minimum) rat chronic/oncogenicity study (00141874) demonstrated a statistically significant treatment-related increased number of female Sprague-Dawley rats with malignant mammary tumors (adenocarcinoma plus carcinosarcoma).

The Toxicology Branch (TB) Peer Review Committee and U.S.E.P.A. Science Advisory Panel (SAP) agree that atrazine is a category C oncogen. In addition, the TB Peer Review Committee is requesting additional studies to determine the most appropriate method, if any, for determining the quantitation of risk due to atrazine (see Issues section for further detail).

There are no toxicologic issues associated with developmental or reproductive studies.

Atrazine is negative in a battery of mutagenicity tests for gene mutation, chromosomal aberration and DNA repair. However, based on published dominant lethal effects, there may be a concern for heritable germ cell effects.

Atrazine is rapidly metabolized by dechlorination of the triazine ring and N-dealkylation. Elimination is also rapid and occurs primarily in the urine and secondarily in the feces. It does not appear to accumulate in the body.

B. Toxicology Profile

81 Series Acute Toxicity and Irritation Studies

81-1 Acute Oral

Sufficient data are available to show that technical atrazine has a low acute oral toxicity in rats (00027097). The acute oral LD_{50} for rats was 2,850 mg/kg for males and females combined. Toxicity Category III.

81-2 Acute Dermal

Sufficient data are available to show that technical atrazine has a low toxicity by the dermal route (00024706). The acute dermal LD_{50} for rats is 1869 mg/kg for males and females combined. Toxicity Category III.

81-3 Acute Inhalation

Although an acute inhalation study on the technical (00059213) indicates that the LC50 for rats is 167 mg/l (nominal), this study had been downgraded to core-invalid since particle size and actual atmospheric concentration and doses were not determined as are currently required by the guidelines. Additional data are required.

81-4 Primary Eye Irritation

Sufficient data are available to show that technical atrazine was moderate eye irritant (ACC231466). Toxicity Category III.

81-5 Primary Dermal Irritation

Sufficient data are available to show that technical atrazine was not a dermal irritant (00027096). Toxicity Category IV

81-6 Dermal Sensitization

Sufficient data are available to show that technical atrazine was not a dermal sensitizer (00105131).

81-7 Acute Delayed Neurotoxicity

No data are available on the acute neurotoxic effects of Atrazine. This test is required only for compounds which are organophosphate inhibitors of cholinesterase, or related to such inhibitors or metabolites of such inhibitors. Atrazine is not an organophosphate, therefore a study is not required.

82 Series Subchronic Testing

82-1 Subchronic Oral

There are no satisfactory subchronic oral studies with atrazine. Studies are not required since there are acceptable chronic feeding studies in both rats and dogs.

82-2 Subchronic Dermal (21-day)

No data are available on the subchronic 21-day dermal toxicity of Atrazine. A study is required.

82-3 Subchronic Dermal (90-day)

There is no subchronic 90-day dermal study. This study is not required because the existing acceptable end-uses should not result in repeated human skin contact for extended periods.

82-4 Subchronic Inhalation

There is no subchronic inhalation study. This study is not required for the registered use patterns.

82-5 Subchronic Neurotoxicity

There is no subchronic neurotoxicity study. This study is not required because a acute delayed neurotoxicity study is not required.

83 Series Chronic and Long Term Studies

83-1 Chronic Toxicity

There is a core-minimum one-year feeding/oncogenicity study (40431301) in the beagle dog (4 dogs/sex/group). They were treated with atrazine in the diet at 0, 15, 150 and 1000 (6 dogs/sex) ppm which resulted in approximate doses of 0, 0.48, 4.97 and 33.65 mg/kg/day for males and 0, 0.48, 4.97 and 33.80 mg/kg/day for females (based on food consumption and body weight). The no-observable-effect-level (NOEL) was 15 ppm (0.48 mg/kg/day) and the lowest-effect-level (LEL) was 150 ppm (0.4.97 mg/kg/day) based on: minimal cardiac changes including dilatation of the right atrium (1 male), thickened atrium with edema consistent with disseminated arteritis (1 male), decreased P-II waves (females). At the high dose, toxicologic effects due to Atrazine included: mortality; cachexia and ascites; decreased body weight, weight gain, and food consumption; electrocardiographic alterations such as irregular heartbeat, increased heart rate, decreased height of the P-II wave, atrial premature complexes and atrial fibrillation; decreased serum protein and albumin; moderate to severe cardiac lesions characterized by atrial dilation, myocardial degeneration (atrophy, myolysis).

This study satisfies the requirement for a chronic dog feeding study.

There is a core-minimum two-year feeding/oncogenicity study (00141874) in the rat (65 rats/sex/group). Rats were treated for up to two years with either 0, 10, 70, 500 or 1000 ppm of Atrazine technical, which results in approximate doses of 0, .5, 3.5, 25 or 50 mg/kg/day (calculated using the .05 conversion factor). There were interim sacrifices at 12 and 13 months. The systemic NOEL was 70 ppm (3.5 mg/kg/day). The systemic LEL was 500 ppm (25 mg/kg/day) based on (males and females): decreased mean body weight. In addition in the high dose females there was decreased survival; decreased hemoglobin, hematocrit and red cell count; increased non-neoplastic pathology (hepatocellular centrolobular necrosis, increased myeloid hyperplasia of the bone marrow). Non-neoplastic pathology in the high dose males included acinar hyperplasia of the mammary gland, renal pelvic calculi and prostatic epithelial hyperplasia. Treatment-related neoplastic pathology was limited to the mammary gland of females. There was a statistical increase in malignant tumors (adenocarcinoma plus carcinosarcomas) and in mammary tumor bearing animals (see toxicological issues for more detail).

This study satisfies the requirement for a chronic rat feeding study.

83-2 Oncogenicity

See "83-1 Chronic toxicity" for comments regarding the 2-year rat study (00141874). This was a core-minimum study for oncogenicity.

This study satisfies the requirement for a rat oncogenicity study.

There is a core-guideline 91-week oncogenicity study (40431302) in mice (59-60 mice/sex/group). They were treated with atrazine in the diet at 0, 10, 300, 1500 or 3000 ppm which resulted in approximate doses of 0, 1.4, 38.4, 194.0 or 383.7 mg/kg/day for males and 0, 1.6, 47.9, 246.9 or 482.7 mg/kg/day for females (based on food consumption and body weight). The systemic NOEL was 300 ppm (45.0 mg/kg/day). The LEL was 1500 ppm (225.0 mg/kg/day) based on: (males and females) decreased mean body weight gain. In addition, cardiac thrombi were observed in female mice at this dose. There was no evidence for oncogenic potential due to atrazine in this study.

This study satisfies the requirement for a mouse oncogenicity study.

83-3 Teratogenicity

There is a core-minimum rabbit teratology study (00143006, 40566301). Groups of 19 New Zealand White rabbits were treated by gavage on days 7 through 19 of gestation with 0, 1, 5 or 75 mg/kg/day of atrazine. The maternal toxicity NOEL was 1 mg/kg/day and the LEL was 5 mg/kg/day based on statistically significant reduction in body weight gain for gestational days 14-19 and a statistically significant reduction in food consumption on gestational days 17 and 19. The developmental NOEL was 5 mg/kg/day and the LEL was 75 mg/kg/day based on increased resorptions, decreased fetal weights and delayed ossification of appendages.

This study satisfied the requirement for a teratology study in rabbits.

There is a core-minimum rat teratology study (00143008, 40566302). Groups of 27 Charles River rats were treated by gavage on days 6 through 15 of gestation with 0, 10, 70 or 700 mg/kg/day of atrazine. The maternal toxicity NOEL was 10 mg/kg/day and the LEL was 70 mg/kg/day based on decreased body weight gain. In addition, at 700 mg/kg/day there was increased mortality, and clinical sings of toxicity along with decreased food consumption. The developmental NOEL was 10 mg/kg/day and the LEL was 70 mg/kg/day based on delayed ossification.

This study satisfied the requirement for a teratology study in rats.

83-4 Reproduction

There is a core-minimum 2 generation reproduction study in rats (40431303). Groups of male and female Charles River (CRCD,VAF/PLUS) rats were exposed to atrazine in the diet at 0, 10, 50 or 500 ppm which results in an approximate dose of 30, 0.5, 2.5 or 25 mg/kg/day (calculated using the .05 conversion factor). The Parental systemic NOEL was 50 ppm (2.5 mg/kg/day) and the LEL was 500 ppm (25 mg/kg/day), based on decreased body weights, body weight gain and food consumption in both parental males and females throughout the study. The reproductive NOEL was 10 ppm (0.5 mg/kg/day) and the LEL was 50 ppm (2.5 mg/kg/day) based on decreased body weights of pups in the second generation at the time of weaning (postnatal day 21.

This study satisfies the requirement for a reproduction study in rats.

84 Series Mutagenicity

84-2 Mutagenicity Tests

1. Gene mutation.

   Atrazine was not mutagenic when tested in S. typhimurium for point mutation (40246601) or E. coli for reverse mutation (00161931).

   The requirements for gene mutation testing have been satisfied.

2. Chromosomal aberration

   There is an acceptable micronucleus test (40722301). When tested in male and female Tif:MAGF,SPF-NMRI-derived mice there was no evidence of micronucleus formation with acute doses up to 2250 mg/kg, a level resulting in death.

   Based on published dominant lethal effects, there may be a concern for heritable germ cell effects (Adler, Mutat. Res. 74: 77-93, 1980). The Agency has obtained summary data from the laboratory for this study that indicate a slight increase in dominant lethal effects at 2000 mg/kg, suggesting that there may be transmissible genetic alterations.

   Although the minimum testing requirement for this mutagenicity category has been formally satisfied, a dominant lethal study is required.

3. Direct DNA damage or other tests.

   Atrazine was tested for DNA repair in primary rat hepatocytes from Tif;RAIf, SPF rats (00161790, 40246602). Although there was no evidence of increased repair both with and without S-9 activation at doses up to 150 ug/ml, this study has been determined to be invalid.

   The requirements for direct DNA damage or other testing have not been satisfied.

The requirements for Mutagenicity testing have not been satisfied.

85 Series Special Studies

85-1 Metabolism

Although there are several acceptable metabolism studies (00080634, 40431304,-5,-6,-9,40437501 there are several issues concerning the metabolism of atrazine that need to be addressed: 1) Identification of fecal metabolites in the male and female rat. 2) Identification of urinary metabolites in the male rat unless an acceptable rationale can be given that the male and female produce the same metabolites. 3) An explanation for the differences between two studies for the percentage elimination of radioactivity in the urine and feces urine of female rats given a single oral dose of 100 mg/kg of atrazine (70:25 vs. 50:50).

Figure 1 presents the structure of the parent and many of the primary metabolites for atrazine in rats. Dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats.

About 95 % of atrazine is eliminated within 7 days following treatment. The whole body half-life is about 1.3 days. Elimination is independent of dose and sex. The primary route of elimination is urine (about 75 %). The amount eliminated in the feces was about 20 %.

Distribution of atrazine was dose dependent but independent of sex. Red blood cells (RBC) appear to store the highest levels radiolabel (through covalent binding of a metabolite). Atrazine did not appear to accumulate in tissue, except possibly for the RBC.

The requirements for Metabolism testing have not been satisfied for the reasons stated above. Additional studies are required.

85-2 Domestic Animal Safety

Studies for domestic animal safety are not required at this time.

85-3 Dermal Absorption

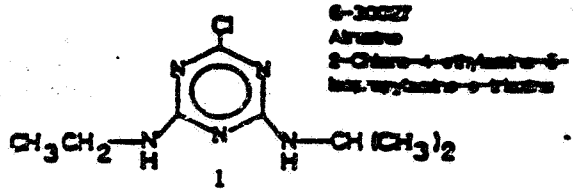
There is an Acceptable dermal absorption study (40431308). Atrazine in 4L formulation is absorbed in relatively small amounts through the skin. Typical values were 2.00, 0.53 and 0.26 % for 10 hour exposures to doses of 0.01, 0.1 or 1.0 mg/cm^2. Significant quantities remain on/in the skin after washing with soap and water (24.87, 21.10 and 10.49 %). No significant differences in absorption were observed between the 4L and 80W formulations tested at 1.0 mg/cm^2 for 10 hours.

The data indicate that absorption is approaching saturation at the high dose. The test material which remains on/in the skin after soap and water wash is considered absorbable. For risk assessments the percent absorbed is added to the percent on/in the skin to determine quantity absorbed. However, as noted in the data evaluation report (DER) it is possible that part the measured atrazine remaining on/in the skin is an artifact of the experimental procedure.

Additional dermal absorption studies are not required at this time.

FIGURE 1

FIGURE I. CHEMICAL NAMES AND STRUCTURES



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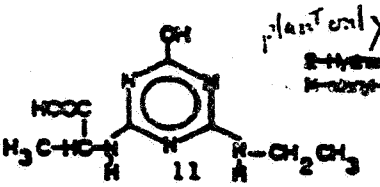

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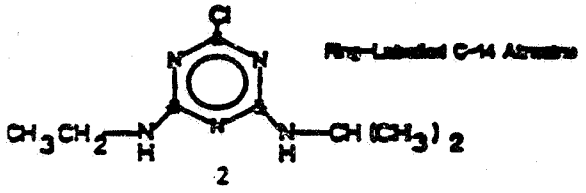

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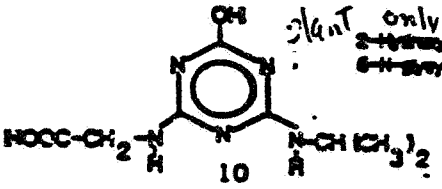
Ring-Labelled C-14 Atrazine



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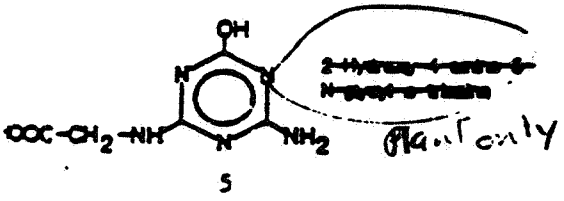

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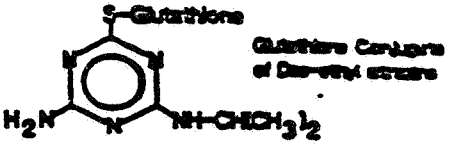

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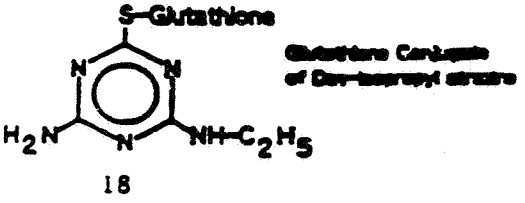

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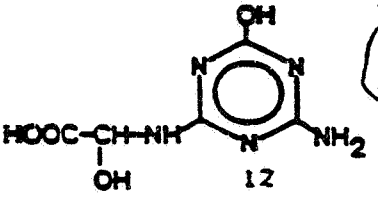

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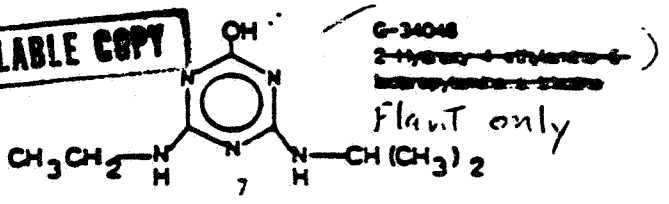

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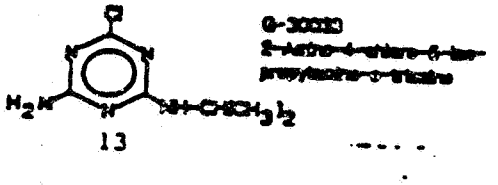

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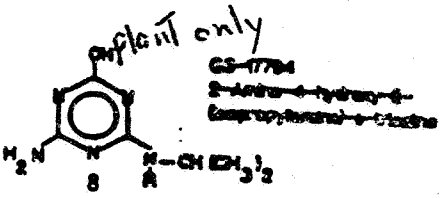

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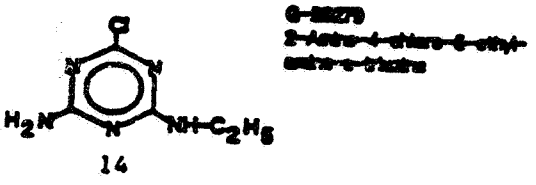

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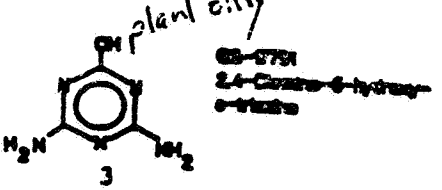

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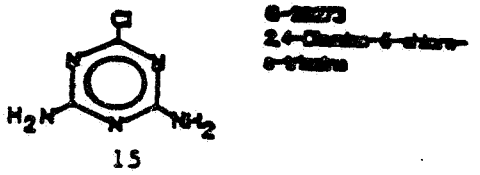

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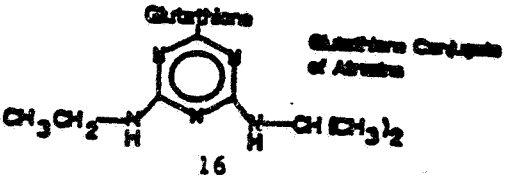

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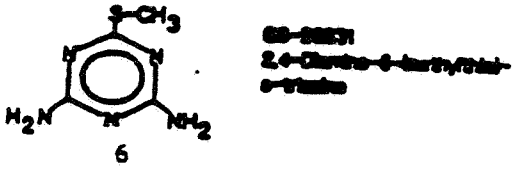

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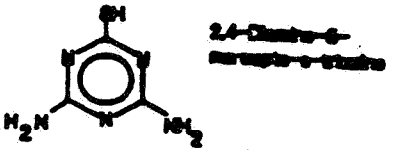

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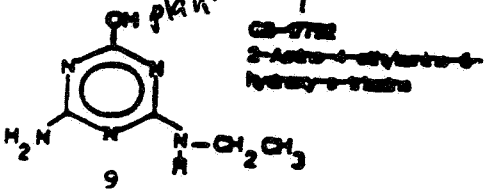

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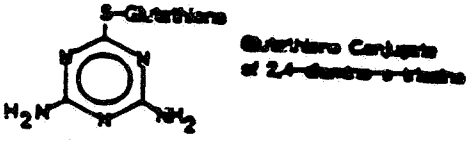

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FIGURE I. CHEMICAL NAMES AND STRUCTURES (Cont.)

C. Data Gaps

Atrazine is registered for use as an herbicide on food crops and therefore the following Guideline toxicology studies can be required for registration.

81 Series
81-1 Acute Oral
81-2 Acute Dermal
81-3 Acute Inhalation
81-4 Primary Eye Irritation
81-5 Primary Dermal Irritation
81-6 Dermal Sensitization
81-7 Acute Delayed Neurotoxicity (hen)

82 Series Subchronic Testing
82-1 Subchronic Oral (rodent, non-rodent)
82-2 Subchronic Dermal (21-day)
82-3 Subchronic Dermal (90-day)
82-4 Subchronic Inhalation
82-5 Subchronic Neurotoxicity

83 Series Chronic and Long Term Studies
83-1 Chronic Toxicity (rodent, non-rodent)
83-2 Oncogenicity (two species)
83-3 Teratogenicity (two species)
83-4 Reproduction (rat)

84 Series Mutagenicity
84-2 Mutagenicity Tests.
1. Gene mutation.
2. Chromosomal aberration
3. Direct DNA damage.
4. Other Tests.

85 Series Special Studies
85-1 Metabolism
85-3 Dermal Absorption

Based on this assessment of the toxicology data base for atrazine, the following Guideline toxicology studies have been identified as data gaps.

81 Series

81-3 Acute Inhalation

82 Series Subchronic Testing

82-2 Subchronic Dermal (21-day)

84 Series Mutagenicity

84-2 Mutagenicity Tests.

Chromosomal aberration - dominent lethal study

Direct DNA damage or other

Based on this assessment of the toxicology data base, the following additional studies are required.

Oncogenicity Study with the Fischer 344 rat (83-2).

Comparative metabolism studies comparing Fischer and Sprague-Dawley female rats (blood and urinary metabolism, blood kinetics and red blood cell binding), and comparisons between other mammalian species (rat hepatocytes).

Hormone/receptor effects studies including evaluation of serum estrogen, corticosterone, progesterone levels (including prolactin if possible).

D. RfD Reassessment

The RfD, previously referred to as the ADI, of 0.005 mg/kg/day is based upon the NOEL of 0.5 mg/kg/day from the chronic dog feeding study and the co-critical study the 2-generation reproduction study. The uncertainty factor of 100 is used to account for the inter- and intraspecies differences. In addition, the Toxicology Branch Peer Review Committee has determined that an additional factor of 10 should be use with the RfD in order to account for the uncertainty due to the oncogenic potential of atrazine.

The data base includes:

1-year feeding - dog
2-year feeding/oncogenic - rat
2-generation reproduction - rat
teratology - rabbit
teratology - rat

Additional data considered: 91-week oncogenic - mouse

There are no data gaps for determining the Rfd. This RfD has been confirmed by the Toxicology Branch and Agency RfD Committees.

E. Toxicological Issues

1. Oncogenicity

Background

Atrazine, although not associated with any serious chronic toxicity in the mouse is associated with tumors in rats. This was determined subsequent to issuance of the RS-83. A core-minimum rat study (0014874) indicated that Atrazine ingestion was be associated with an increased incidence of malignant mammary tumors in female but not male rats.

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] were considered when conducting a weight-of-the-evidence determination of oncogenic potential.

The high dose (1000 ppm - 50 mg/kg/day) reached or exceeded the adequate dose needed for assessment of oncogenic potential based on: males - 25 % decrease in body weight gain and non-neoplastic pathology in males; females - increased mortality, 26 % decrease in body weight gain and non-neoplastic pathology. The 500 ppm (25 mg/kg/day) dose reached an adequate level based on a decrease in body weight gain of 17 and 18 % for males and females, respectively. There was no treatment related systemic toxicity at 70 and 10 ppm (3.5 and 0.5 mg/kg/day).

Dietary administration of Atrazine was associated with malignant mammary tumors in only one species (rat), and only 1 sex (female). Table 1 presents the tumor data using effective proportions (this adjusts for the occurrence of the first tumor). All statistics were conducted with these values, including the Peto prevalence since there was a survival disparity. Table 2 presents the tumor data as crude proportions (no adjustment for occurrence of first tumor). Table 3 presents historical control values from the testing facility (as crude proportions).

- As can be seen in Table 1, there was a statistically significant dose-related trend in females for both malignant and all mammary tumor bearing rats ($p < 0.01$).
- There was also pair-wise significance at the 3.5 and 25 mg/kg/day ($p < 0.05$) and 50 mg/kg/day ($p < 0.01$).
- As can be seen in Table 2, both malignant and TBA values for 3.5, 25 and 50 mg/kg/day are well outside the range for historical controls. Low dose values are only slightly outside the historical control range and probably do not reflect a treatment related increase.

Although the 25 and 50 mg/kg/day doses appeared to produce adequate to excessive toxicity in females, malignant and all mammary tumor bearing rats were also significantly increased at 3.5 mg/kg/day, without excessive toxicity. There was also an

indication that adenocarcinomas occurred earlier in the high dose than in controls. At the 13 week sacrifice there were 5 out of 10 rats with this tumor while none occurred in ten controls. This suggests a possible increase in rate of tumor occurrence. There was also an increase in the proportion of malignant tumors.

Atrazine was not mutagenic in several acceptable studies (in vitro point mutations assays (bacterial); DNA repair test; and micronucleus test). Although there are several questionable published positive studies the mutagenic data does not, in this case affect the oncogenic classification.

Atrazine is structurally related to several compounds of known oncogenic potential.

Risk Assessment

The Peer Review Committee documents 1 and 2 (dated 3/1/88, and 7/7/88, respectively) indicated that the above constituted only limited evidence of oncogenicity since the oncogenic response was observed only in 1 species (rat) and sex (female) and was a common tumor type (mammary) and classified Atrazine as a Group C, Possible Human Carcinogen. Additional evidence from short-term tests were not supportive of a higher classification. They also concluded that since there was a: 1) possible increase in proportion of malignancy, 2) structural relationship to other known oncogens and 3) possible early onset of tumors, the data warranted quantitative risk assessment using the Q_1^* derived from the Weibull model.

On September 7, 1988, Atrazine was presented to the SAP for consideration of the classification. They agreed with the conclusions of the TB Peer Review Committee that Atrazine is a class C oncogen. Although they stated that the mammary tumors in Sprague-Dawley rats should be considered as a biologically significant endpoint, they did not agree that quantification of risk should be performed.

As a result of reevaluating the classification and quantification in response to the Scientific Advisory Panel, the Peer Review (document 3) determined that:

> "the data for Atrazine are not appropriate for quantitative risk assessment (using the Weibull model). This was because the tumors occurred only in one sex (female), species (rat) and strain (Sprague-Dawley). Mammary tumors occur at a variable rate, with a high background incidence in this strain of rat. Additional mutagenicity data have lessened the concern for this endpoint, therefore genotoxicity could not be used to support quantification. There appears to be, although not yet substantiated by data, a hormonal mechanism."

In addition the Peer Review Committee felt that since the tumors were of toxicologic concern, the RfD, as used by the Office of Pesticide Program, was inadequate. It was concluded that until such time as additional data (see below) was presented to support an appropriate model for quantitation, an additional uncertainty factor of 10 should be added to the RfD, which usually has an uncertainty factor of 100. It was also their intent that, when other routes of exposure are evaluated for levels of concern, that the uncertainty factor of 100 be increased by a factor of 10. This would result in a Margin of Exposure level of 1000, below which there is toxicologic concern for oncogenicity. The Office of Drinking Water has used this additional factor of 10 when determining the Lifetime Health Advisory (LHA) for atrazine in drinking water.

Data Needed to Reevaluate the Appropriate Risk Characterization Model (taken from the TB Peer Review Document 3)

> "The listed studies are currently ongoing, or are in the planning stages at Ciba-Geigy. Preliminary results were pivotal in the above deliberations of the Peer Review Committee. Data provided by the completed studies are necessary for the Committee to determine the most appropriate method, if any, for quantifying the risk due to atrazine:
>
> 1) Hormone/receptor effects of Atrazine, including serum estrogen and other endocrine levels (including prolactin if possible);
>
> 2) Comparison of oncogenic potential between Fischer and Sprague-Dawley female rats;
>
> 3) Comparative metabolism between Fischer and Sprague-Dawley female rats, and between other mammalian species (rat hepatocytes."

2. **Mutagenicity - concern for heritable risk**

The minimum regulatory requirements for chromosomal aberration testing have been fulfilled at this time. However, based on the positive results in a published dominant lethal study, there may be a concern for heritable germ cell effects (Adler, Mutat. Res. 74: 77-93, 1980) from atrazine exposure. It should be noted that since these results are not overwhelmingly positive, they alone would not suggest a high priority concern for heritable risk. However, due to the high potential for human exposure to atrazine, further examination is warranted.

It is requested that an acceptable dominant lethal assay with male mice be performed with atrazine active ingredient. Since it is the intention to reproduce the published study's results, it

TABLE 1 Rate[1] of female mammary tumors in the 2-year dietary oncogenicity study in female rats (Atrazine) (Am.Biogen. #410-1102, 4/29/86)

EFFECTIVE PROPORTIONS[1]

Mammary tumor	DOSE 0	10	70	500	1000
Benign[2] only	20/88(23)	24/65(37)	21/69(30)	21/68(31)	20/89(22)
Malignant[3,4]	15/88**(17)	16/67(24)	27/69*(39)	27/68*(40)	45/89**(51)
TBA[5]	35/88**(40)	40/67(60)	48/69*(70)	48/68*(71)	65/89**(73)

NOTE: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.
* $p<0.05$; ** $p<0.01$ (Peto prevalence test)
1 Animals with tumor/animals alive after occurrence of first tumor.
2 Fibroadenoma and/or adenoma.
3 All adenocarcinoma plus carcinosarcoma.
4 Two animals in the high dose group had carcinosarcomas.
5 Mammary tumor bearing animals.

is strongly recommended that the registrant discuss their proposed protocol(s) (e.g. dose selection and range, vehicle, animal strains) with the OPP.

The Agency has obtained summary data (from the testing laboratory) for the published study that indicate a slight increase in dominant lethal effects in mice at 2000 mg/kg. This suggests that there may be potential transmissible genetic alterations (see memorandum from Kerry Dearfield to Marion Copley dated 12/8/88 for additional detail). "There was a low frequency of fertile matings in the first mating period (38.6 % compared to 96 % frequency for controls). However, once pregnant, females appeared to have a comparable number of corpora lutea and implants as the controls. There was a slight increase in dominant lethal mutations in the first 3 mating periods (i.e. first 12 days mating post-treatment) as evidenced by an increase in the percent dead implants over controls." This study used olive oil as the vehicle. In an unacceptable dominant lethal study conducted by Ciba-Geigy Ltd. in a different strain of mice (40246603), there was no evidence of dominant lethal effects. This study remained unacceptable due to the lack of toxicity at the high dose (1332 mg/kg) despite the collection of additional information. The vehicle in this study was carboxymethyl cellulose. This may be a contributing factor to the difference in the results of these two studies.

TABLE 2 Rate[1] of female mammary tumors in the 2-year dietary oncogenicity study in female rats (Atrazine) (Am.Biogen. #410-1102, 4/29/86)

CRUDE PROPORTIONS[1]

Mammary tumor	DOSE 0	10	70	500	1000
Benign[2] only	20/88(23)	24/69(35)	21/69(30)	21/70(30)	20/89(22)
Malignant[3,4]	15/88**(17)	16/69(23)	27/69*(39)	27/70*(39)	45/89**(51)
TBA[5]	35/88**(40)	40/69(58)	48/69*(70)	48/70*(69)	65/89**(73)

NOTE: Stars are for reference only,they refer to the previous table.

TABLE 3 HISTORICAL CONTROLS (273 rats at Am. Biogen. Corp.)

Mammary tumors	mean % from 4 studies (range)
Benign[2] only	36 (31-45)
Malignant[3,6]	10 (3-19)
TBA[5]	45 (40-51)

1 Animals with tumor type/# examined (percent).
2 Fibroadenoma and/or adenoma.
3 All adenocarcinoma plus carcinosarcoma.
4 Two animals in the high dose group had carcinosarcomas.
5 Mammary tumor bearing animals.
6 There were no carcinosarcomas in the historical controls.

3. Cardiac Toxicity

Background

Atrazine has been shown to produce cardiotoxicity in a 1 year feeding study in dogs (40431301). At the mid dose of 5.0 mg/kg/day there were minimal cardiac changes including dilatation of the right atrium (1/4 males), thickened atrium with edema consistent with disseminated arteritis (1/4 males), decreased P-II waves (4/4 females). At the high dose (34 mg/kg/day), cardiotoxic effects included: electrocardiographic alterations such as irregular heartbeat, increased heart rate, decreased height of the P-II wave, atrial premature complexes and atrial fibrillation; and moderate to severe histopathologic cardiac lesions characterized by atrial dilation, myocardial degeneration (atrophy, myolysis). Related signs of toxicity at this dose included: mortality; cachexia and ascites; decreased body weight, weight gain, and food consumption; and decreased serum protein and albumin. Due to the life threatening potential as evidenced by these cardiac lesions at the high dose, the mid dose is considered to be the lowest effect level even though the lesions at this dose were of much lower magnitude and frequency.

Risk Assessment

Chronic dietary exposure is evaluated using the RfD (discussed earlier in this chapter) while subchronic applicator risk for this toxic endpoint is evaluated by a MOS (margin of safety). The MOS is derived by the following equation:
MOS = NOEL/daily exposure. The NOEL of 0.5 mg/kg/day, is based on cardiac toxicity in the dog chronic feeding study (40431301) and the exposure is the actual daily exposure to the applicator taking dermal absorption into account. While it may be considered extreme to use an endpoint from a chronic study, it is deemed appropriate for the following reasons:

1) An acceptable subchronic dog study is not available.
2) The supplementary 90-day dog study (00163339) that is available, does not have a NOEL established at the low dose (5 mg/kg/day) due to decreased bodyweight gain in males.
3) Although there were no histologic cardiac effects observed in this study, electrocardiography was not conducted. This parameter appears to be the most sensitive indicator of cardiotoxicity in the dog.

Until such time as an appropriate subchronic study is conducted, the chronic NOEL for the dog should be used for determining the MOS for cardiotoxicity.

MOSs of less than 100 are considered to be of toxicologic concern depending upon the number of days of exposure per year.

F. Toxicology Summary Tables

TABLE A
GENERIC DATA REQUIREMENTS FOR ATRAZINE

Data Requirement	Composition[1/]	Use Patterns[2/]	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partial)[3/]	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)[4/]
Tier II Toxicology					
ACUTE TESTING					
81-1 - Acute Oral - Rat	TGAI	A,B	YES	00027097	NO
81-2 - Acute Dermal	TGAI	A,B	YES	00027097	NO
81-3 - Acute Inhalation - Rat	TGAI	A,B	NO		YES
81-4 - Eye Irritation - Rabbit	TGAI	A,B	YES	ACC231466	NO
81-5 - Dermal Irritation - Rabbit	TGAI	A,B	YES	00027096	NO
81-6 - Dermal Sensitization - Guinea Pig	TGAI	A,B	YES	00105131	NO
81-7 - Acute Delayed Neurotoxicity - Hen	TGAI	A,B	NO		NO[1/]
SUBCHRONIC TESTING					
82-1 - 90-day Feeding - Rodent	TGAI	A,B	NO		NO[5/]
Non-rodent	TGAI	A,B	NO		NO[5/]
82-2 - 21-Day Dermal -	TGAI	A,B	NO		YES

Data Requirement	Composition[1]	Use Pattern[2]	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)[3]
83 Wildlife Toxicology (Continued)					
83-3 - 90-Day Dermal -	TGAI	A,B	NO		NO[6]
83-4 - 90-Day Inhalation -	TGAI	A,B	NO		NO[7]
83-5 - 90-Day Neurotoxicity -	TGAI	A,B	NO		NO[8]
CHRONIC TESTING					
83-1 Chronic Toxicity					
Rat	TGAI	A,B	YES	00141874	NO
Dog	TGAI	A,B	YES	40431301	NO
83-2 - Oncogenicity Study -					
Rat	TGAI	A,B	YES	00141874	NO
Mouse	TGAI	A,B	YES	40431302	NO
83-3 - Teratogenicity -					
Rabbit	TGAI	A,B	YES	00143006 40566301	NO
Rat	TGAI	A,B	YES	00143008 40566302	NO

Data Requirement	Composition[1]	Use Patterns[2]	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)[3]
82 - 85 Toxicology (continued)					
83-4 - Reproduction	TGAI	A,B	YES	40431303	NO
MUTAGENICITY TESTING					
84-2 - Gene Mutation	TGAI	A,B	YES	00161931 40246601	NO
84-2 - Chromosomal Aberration	TGAI	A,B	PARTIALLY	40722301	YES[9]
84-2 - Other Mechanisms of Mutagenicity	TGAI	A,B	NO		YES
SPECIAL TESTING					
85-1 - General Metabolism	PAI or PAIRA	A,B	PARTIALLY	40431304 40431305 40431306 40431309 40437501 00080634	YES[10]
85-3 - Dermal Absorption	PAI or PAIRA	A,B	YES	40431308	NO

Data Requirement	Composition[1/]	Use Pattern[2/]	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)[3/]
40 CFR Toxicology (continued)					
SPECIAL ADDITIONAL STUDIES					
Oncogenicity, Study - Fischer 344 rat	TGAI		NO		YES[11/]
Comparative metabolism -	TGAI		NO		YES[12/]
Hormone/receptor effects -	TGAI		NO		YES[13/]

1/ Composition: TGAI = Technical Grade Active Ingredient; PAI = Pure Active Ingredient; PAIRA = Pure Active Ingredient, Radiolabelled, Choice = Choice of several test substances determined on a case-by-case basis

2/ The use patterns are coded as follows: A = Terrestrial, Food Crop; B = Terrestrial, Non-Food; C = Aquatic, Food Crop; D = Aquatic, Non-food, E = Greenhouse, Food Crop; F = Greenhouse, Non-Food; G = Forestry; H = Domestic Outdoor, I = Indoor, IP = Industrial Preservative.

3/ Unless otherwise specified, data must be submitted no later than six months after publication of this Standard.

4/ An acute neurotoxicity study is not required since atrazine is not an organophosphate.

5/ This requirement is waived based on the submission of an acceptable chronic feeding study.

6/ This study is not required because existing acceptable end-uses should not result in repeated human skin contact.

7/ This study is not required because existing acceptable end-uses should not result in repeated inhalation exposure.

8/ This study is not required because an acute delayed neurotoxicity is not required.

9/ Although the minimum testing requirement for this mutagenicity category has been formally satisfied, a dominant lethal study (as described in the Toxicology Chapter of this standard) is required (due date 12 months).

10/ The following metabolism data are needed: 1) Identification of fecal metabolites in male and female rats; 2) Identification of urinary metabolites in male rats; 3) justification for the marked difference when comparing the percent radioactivity eliminated in feces and urine in two independent studies

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1/ Due date January 1990.

2/ This includes comparing Fischer and Sprague Dawley female rats (blood and urinary metabolites, blood kinetics and rat blood ce... binding), and comparisons between other mammalian species (rat hepatocytes). Due Jan. 1989.

3/ This includes both estrogen, corticosterone, progesterone levels (including prolactin if possible). Due April 1989

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G. Bibliography

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00163339 Tisdel, M.; Harris, D. (1977) 90-Day Subacute Feeding Study of Atranex in Dogs: Final Report: Study No.: T-635. Unpublished study prepared by WARF Institute. 155 p.

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40431301 O'Connor, D.; McCormick, G.; Green, J. (1987) Chronic Toxicity Study in Dogs: Atrazine Technical: Laboratory Study No. 852008. Unpublished study prepared by Ciba-Geigy Corp. 1405 p.

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H. One Liners

TOXCHEM NO. 063 · Atrazine

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS		TOX CAT	CORE GRADE/ DOCUMENT#
Primary eye irritation Species: rabbit Institute Di Ricerche 12/76	Atrazine Tech.	231466	Corneal opacity and conjunctivitis were present up to and including 72 hrs.		2	Minimum 000538 006937
Acute Dermal LD50 Species: rat Consultox Lab Ltd. CL 74:46:996E; 4/74	Atrazine tech. 95% a.i.	00027097	LD50 > 2000 mg/kg. No toxicity noted.		3	Minimum 002917 006937
Acute oral LD50 Species: rat Consultox Lab Ltd. CL 74:46:996E; 4/74	Atrazine Tech. 95% a.i.	00027097	LD50 = 2850 mg/kg		3	Minimum 002917 006937
Dermal irritation - rabbit; Hazleton Labs. Am., Inc.; #915-102; 3/5/75	Technical Purity not reported	00027096 (MRID)	Non irritating	IV		Minimum 0002917 006937
Acute inhalation LC_{50} - rat; Hazleton Labs. Am. Inc., #915-100; 4/1/75	Technical Purity Not Reported	00027095 (MRID)	LC_{50} > 167 mg/L/1 hr - nominal concentration. Purity not stated No actual or atmospheric exposures	X		Minimum 002917 Invalid 006937
Dermal sensitization - guinea pig; Wil; #Wil-1214-78; 3/21/79	Atrazine 95%	00105131	Nonsensitizing			Minimum 002816 006937
90-day - dog/WARF Nutr. Tox Facility T635/1977	Atrazine Technical	00163339	Fed at 0, 200, 632, 2000 ppm in diet (0, 5, 15.8, 50 mg/kg/day) in beagles. NOEL < 200 ppm (5 mg/kg/day) (10T) LEL ≤ 200 ppm based on body weight gain depression in males. In addition at 623 ppm and above in males, there was a slight decrease in RBC, HCT and HGB. There was also a mild to Total arrest of Spermatogenesis. AT 2000 ppm in males there was decreased food consumption; in females there was body weight loss decreased food consumption, and a slight decrease in RBC, HCT			Supplementary 006937

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	CORE GRADE/ DOC NO.
Oncogenic Species: mice [illegible] [illegible]; 6/30/81	Atrazine tech		IBT · Supplementary ; Dynamac Corp. ; Accepted by EPA 5/31/83		Supplementary 003183 006937
Feeding/oncogenic 2 year Species: rat American Biogenics Corp. 410 1102; 4/29/86	Atrazine 98.9% Tech.	262714- 262727 00[illegible]	Doses tested: 10, 70, 500, 1000 ppm in CD-1 Sprague Dawley. Oncogenic NOEL = 10 ppm. Oncogenic LEL = 70 ppm. Females: there were sta. sig. increases in carcinomas for females receiving 70, 500 & 100 ppm of atrazine; in adenomas & fibroadenomas for fem. receiving 1000 ppm & in total mammary tumors in females receiving 500 & 1000 ppm. There was sig. pos. dose trends for all 3 categories (sarcomas, fibroadenomas plus		Minimum 005960 006937
Feeding/Oncogenic 22 months Species: mice Ciba Geigy Ltd. A-2120, 10/30/87	Atrazine, batch 041002	404313-02	Levels tested in Charles river CD 1(Crl:CD(ICR)BR) str: 0, 10, 300, 1500 and 3000 ppm. Oncogenic NOEL > 3000 ppm. Systemic NOEL = 300 ppm Systemic LEL = 1500 ppm (decr of 23.5 and 11% in body wt. of ♂ & ♀; incr. incidence of cardiac thrombi in females).		Guideline 006718 006765 006937
Feeding 1 year Species: dog Ciba Geigy Ltd [illegible] 10/27/87	Atrazine Tech. (97%)	404313-01	Doses (ml): 0, 15, 150, 1000 ppm (equivalent to intakes of 0, 0.48, 4.97 and 33.65/33.80 M/F mg/kg/day). NOEL = 15 ppm (0.48 mg/kg/day). LEL = 150 ppm (4.97 mg/kg/day) (Cardiac changes).		Minimum 006718 006937
Teratology Species: rat Ciba Geigy Pharmaceutical, Eng [illegible], 9/18/84	Atrazine tech.; 96.7%	256979 00[illegible]	Maternal NOEL = 10 mg/kg. Maternal LEL = 70 mg/kg (reduced body wt. gain in 1st half of gestation). High mortality at 700 mg/kg (HDT). Developmental NOEL = 10 mg/kg. A/D ratio = 1 (10/10) Developmental LEL = 70 mg/kg (delayed ossification). Doses: 10, 70, 700 mg/kg/day.		Supplementary 006131 Minimum 006761 006937
Teratology Species: rabbit Ciba Geigy Pharmaceutical, Eng [illegible], 9/18/84	Atrazine tech. 96.3%	256979 0014 5066 405[illegible]630	Maternal NOEL = 1 mg/kg/day. Maternal LEL = 5 mg/kg/day. (reduced body wt. gain & red. food consump). Develop. NOEL = 5 mg/kg/day. Develop LEL = 75 mg/kg/day (incr. resorptions, decr. fetal wts of male & female pups, delayed ossification of [illegible]). A/D ratio = 0.2 (1/5) Doses: 0, 1, 5, 75 mg/kg/d in N.Z.W. str by gavage on 7-19d gestation.		Supplementary 006131 Minimum 006761 006937
Reproduction 2 generation Species: rat Ciba Geigy Pharmaceutical, Eng [illegible], 11/17/87	Atrazine Tech.	404313-03	Dosage levels: 0, 10, 50, 500 ppm. Parental NOEL = 50 ppm Parental LEL = 500 ppm based upon decr. body wts, body wt. gain, and food consumption in both parents and females throughout the study. In addition the increase in relative testes weight seen in parental males could be treatment related since it was seen in both generations. Reproductive NOEL = 10 ppm. Reprod. LEL = 50 ppm based upon decr. body		Minimum 006718 006937
Mutagenic-dominant lethal test Species: mice Ciba-Geigy Ltd., Switz. 801380; 9/8/81	Atarzine Tech 98.9% lot 6663	284052 40 24602	Male mice were gavaged with 444 or 1332 mg/kg; gave no toxic findings. Unable to assess gonad exposure.		Unacceptable 005833

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	CORESANDC/ DOCUMENTS
Mutagenic DNA repair test Species: rat hepatocytes Ciba Geigy Ltd., Switz. 831171; 5/10/84	Atrazine Tech; Batch # P210200; 98.2%	282052 [redacted] 402466-02 00161790	Primary rat hepatocytes from fif; RAIf, SPF, were exposed to 1.2, 6, 30 and 150 ug/ml of atrazine w/o S-9 activation. Radio thymidine uptake was not evidently greater than controls. However background counts were not carried out. unacceptable 006917		Unacceptable 005833 Acceptable 006368 006937
Mutagenic In Vitro Species: microbes Nomura Research Inst. (Japan) RRI /V 2884; 8/79	Atrazine Lot G30027 98.8%	284052 00161931	B. subtilis (rec assay) only unactivated; neg. for genotoxic effect. E. coli (rev. mut.); 50, 100, 500, 1000, 2000, 5000 ug/plate w/wo S-9 activation. No incr. in reverse mut. rates. S. typh. (rev. mut.); 50 to 10,000 ug/plate w/wo S-; control values too high.		Unacc (B. subtil) 005833 Accep (E.coli) 005833 Unacc (S.typh) 005833 006937
Mutagenic Ames Species: Salmonella Ciba Geigy Corp. Inc. 861172; 12/5/86	Atrazine G30027 Tech 98.2%; lot 210200	264052 402466-01	Tested at 20, 78, 313, 1250 & 5000 ug/plate with/without activation. Negative. No increase in histidine-prototrophic mutants both with and without metabolic activation.		Acceptable 006368 006937
Mutagenic micronucleus assay Species: mice Genetic Toxicol. Lab 871546; 5/31/88	Atrazine Tech. (92.2%)	407223-01	Negative for induction of micronuclei in mice treated orally at acute doses up to a level causing death (2250 mg/kg).		Acceptable 006917 006937
Dermal absorption Species: rat Ciba Geigy Ltd. ABR 87098; 11/6/87	Atrazine-C14 labelled	404313-08	Atrazine in 4L formulation is absorbed in relatively small amts through the skin. Typical values are 2.00, 0.53 and 0.26 % for 10 hr. exposure to doses of 0.01, 0.1 or 1.0 mg/cm2. Significant quantities remain on the skin after washing with soap and water (24.87, 21.10 and 10.49%). No significant differences in absorption were observed between the 4L & 80W formulations tested at 1.0 mg/cm2 for 10 hrs. The data indicate that		Acceptable 006718 006937
Metabolism Species: rat Ciba Geigy Ltd. ABR 87087; 10/23/87	Atrazine-C14	404313-05	The distribution of atrazine in rats was found to be dose dependent and to follow first order kinetics. Of the tissues studied, the red blood cells store the highest levels of atrazine, apparently through the covalent binding of a metabolite. In rats exposed to a dose of 100 mg/kg atrazine for 10 days, in decreasing order, the levels found in the following tissues were: red blood cell, liver, kidney, ovary, pituitary, brain,		Acceptable 006718 006937
Metabolism Species: rat Ciba Geigy Ltd. ABR 87115; 10/17/87	Atrazine-C14	404313-06	The characterization & identification of a number of urinary atrazine metabolites in the female Sprague Dawley rat was reported. The data in this study indicates that N-dealkylation is the major metabolic pathway for atrazine in rats. Oxidation of the alkyl substituents of atrazine appears to be a minor and secondary metabolic route.		Acceptable 006718 Acceptable 006751 006937
Metabolism Species: rat Ciba Geigy Ltd. ABR 87116; 11/17/87	Atrazine C14	404575-01	The whole body half life of 1.61 days for atrazine is consistent with the observation that 95% of the administered dose is eliminated within 7 days after exposure. The red cells store the highest concentration of atrazine in the rat, apparently through covalent binding of a metabolite. In rats given repeated daily oral doses of 1 mg/kg atrazine, in decreasing order the levels found in the following tissues were: red blood cells, liver,		Acceptable 006718 Acceptable 006751 006937

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	CORE GRADE/ DOCUMENT
Metabolism Species: rat (Sprague Dawley) Ciba-Geigy Ltd. Acc 87116; 10/23/87	Atrazine C14	404313 04	The whole body half-life of 1.30 days for atrazine is consistent with the observation that about 95% of the administered dose is eliminated within 7 days after exposure. About 75% of the atrazine is excreted through the urinary route whereas about 20% of the atrazine is eliminated in the feces. The elimination route for the remaining 5% was not reported. The red cells store the highest conc. of atrazine in the rat, apparently		Acceptable UNK/UNK 006937
Metabolism Species: rat Nutrition 7/15/80	atrazine - 14C	00080634	67% - 72% of the radioactivity was eliminated within 48 hrs via the urine and feces . Metabolites not determined ; blood levels =2 x that in tissues ; adequate only as an excretion study		Minimum UNK/UNK Minimum UNK25 006937

I. Peer Reviews of Atrazine

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1. First Peer Review of Atrazine (3/1/88)

FILE COPY

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

006037

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Peer Review of Atrazine

From: Judith W. Hauswirth, Ph.D. Judith W Hauswirth 5/1/88
Section Head, Section VI
Toxicology Branch/HED (TS-769C)

To: Robert Taylor/Clare Grubbs
Product Manager #25
Registration Division (TS-767C)

The Toxicology Branch Peer Review Committee met on September 10, 1987 to discuss and evaluate the weight of the evidence on atrazine, with particular reference to its oncogenic potential.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with peer review unless otherwise stated).

Theodore M. Farber — Theodore M. Farber
William Burnam — Wm E Burnam
John A. Quest — John A Quest
Esther Rinde — [illegible]
Richard Levy — [illegible]
Donald Barnes — Donald E. Barnes
Judith W. Hauswirth — Judith W. Hauswirth

2. Peer Review Committee Members in Absentia: (Committee members who were not able to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Reto Engler — R G Taylor
Robert Belliles — Robert Belliles
Anne Barton —
Diane Beal — Diane G Beal

B. Material Reviewed:

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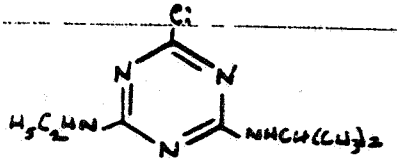
The material available for review consisted of a package prepared by Dr. Hauswirth containing data evaluation of a rat oncogenicity study, an interim report on a IARC rat study, statistical analysis of rat mammary gland tumor data, historical control data and Toxicology Branch "one-liners".

C. Background Information:

Atrazine is a selective herbicide used for season-long weed control in corn, sorghum and other crops. It is used at the highest rate for non-selective weed control in noncropped areas.

The chemical name of atrazine is 2-chloro-4-ethylamino-6-isopropylamino-S-triazine. The structure is as follows:



A peer review of the atrazine data was requested by the Office Director of OPP in the absence of a mouse oncogenicity data because of a possible ground water contamination problem with atrazine.

D. Evaluation Oncogenicity Studies:

1. Rat Oncogenicity Study - Ciba Geigy

Ref.: Twenty-four month combined chronic oral toxicity and oncogenicity study in rats utilizing atrazine technical. Mayhew, DA, Taylor, CD, Smith, SH and Banas, DA. Conducted by American Biogenics Corporation for Ciba-Geigy Corp. Study No. 410-1102. Accession No. 262714-262727. April 29, 1986.

Sprague-Dawley [Crl:CORS CD(SD)BR] rats were started on diets containing atrazine at 37-38 days of age. The dosage levels of atrazine used for the chronic toxicity and oncogenicity portions of the study were 0, 10, 70, 500 and 1000 ppm. Twenty rats per sex per group were used for the chronic toxicity group, i.e. rats used to measure blood parameters and clinical chemistries and urinalysis. Fifty rats per sex per group were used for the oncogenicity study and were maintained on diets for 24 months. An additional 10 rats per sex were placed on control and high dose (1000 ppm) diets for a twelve month interim sacrifice and another 10 per sex (control and high dose, 1000 ppm) for a 13 month sacrifice (the 1000 ppm group was placed on control diet for one month prior to sacrifice). The total number of animals/sex in the control and HDT groups was 90 and 70 for the 10, 70, and 500 ppm groups. Histopathology was performed on all animals.

The incidence of relevant neoplastic pathology seen in this study can be found summarized in Table 1.

Incidence of Relevant Neoplastic Pathology

Tissue	Dose (ppm) 0	10	70	500	1000
			Females		
Mammary Gland					
fibroadenoma[1]	29/89**(32)	29/65(45)	36/70(51)	39/68(57)	45/88**(51)
fibroadenoma[2] (only)	20/79(25)	24/65(37)	21/69(30)	21/66(32)	19/75(25)
adenoma[1]	1/57(2)	0/51(0)	1/57(2)	1/54(2)	2/50(4)
adenoma[2] (only)	1/45(2)	0/43(0)	0/49(0)	0/42(0)	1/33(3)
adenocarcinoma plus carcinosarcoma[3]	15/90**(17)	16/68(23)	27/70*(39)	27/68*(40)	45/90**(50)
TBA[4]	36/90**(40)	40/68(59)	47/70(67)	48/68(71)	65/90**(72)
			Males		
Testes interstitial cell tumor	1/58*(2)	3/59(5)	2/59(3)	2/60(3)	7/64*(11)

Note: Significance of trend denoted at Control.
Significance of pairwise comparison with control denoted at Dose level.
*p<0.05, **p<0.01.

1 Including animals that may also have a carcinoma
2 Excluding animals that may also have a carcinoma
3 Two animals in the high dose group had carcinosarcomas.
4 Total tumor bearing animals - # of animals with at least one type of mammary tumor.

Tumors were analyzed by Peto Prevalence since survival differences were observed in both male and female rats.

The following tumor incidences were statistically significantly elevated in atrazine treated rats:

1. In females:

a. The incidence of fibroadenomas (including animals that may also have a carcinoma) was increased at the high dose. This increase was associated with a significant dose-related trend;

b. The incidence of adenocarcinomas (including two rats at the high dose with carcinosarcomas) was increased at 70, 500, and 1000 ppm. This increase was associated with a significant dose-related trend; and

c. The total number of rats with at least one type of mammary tumor was increased at the high dose. This increase was associated with a significant dose-related trend.

2. In males, the incidence of testicular interstitial cell tumors was increased at the high dose. This increase was associated with a significant dose-related trend (driven by a high dose effect).

There were also indications of reduced latency of mammary gland adenocarcinomas, since at the 12 month interim kill there were 0/20 adenocarciomas in the control group and 6/20 in the high dose group.

The historical control range for fibroadenomas was reported by the performing laboratory to be 36-48%. The incidence of fibroadenomas at the high dose in the atrazine study was just outside of the historical control range. The incidence of adenocarcinomas was outside of the historical control range (3-19%) for all treated groups. The number of animals with mammary gland tumors of any type was also outside of the historical control range (28-51%) for all treated groups. The incidence of testicular interstitial cell tumors was within the historical control range (0-12%).

The Committee felt that the MTD had been exceeded in female rats at 1000 ppm based upon a statistically significant increase in mortality, a 26% decrease in body weight gain at week 13 of the study, and non-neoplastic pathology including a significant increase in centrolobular necrosis of the liver and bone marrow myeloid hyperplasia. The MTD was reached at 500 ppm based upon body weight gain decrement of 18% at week 13 and an increase in bone marrow myeloid hyperplasia.

In male rats, the MTD was exceeded at 1000 ppm based upon a 25% decrease in body weight gain at week 13 of the study which remained at this same percentage decrement for the remainder of the study. Other non-neoplastic pathology seen at 1000 ppm was mammary gland acinar hyperplasia, kidney pelvic calculli, epithelial hyperplasia of the prostate and degeneration of muscle. As was seen in females, the MTD was reached at 500 ppm due a 17% decrease in body weight gain.

2. Chronic Feeding/Oncogenicity Study in Fischer 344/LATI rats conducted for IARC:

Since the full report of this study was not available for the Committee's review it was not considered in the weight-of-the-evidence for atrazine.

E. Additional Toxicology Information:

1. Mutagenicity:

Atrazine was negative in the following acceptable assays used to determine mutagenic potential: Ames Salmonella assay, rec assay in Bacillus subtilis, reverse mutation in E. coli and unscheduled DNA synthesis assay in rat hepatocytes.

2. Reproduction and Teratology:

Atrazine is not teratogenic in the rat or rabbit. In the rat, an increased incidence of runts was seen at all dosage levels (NOEL<10mg/kg). In the rabbit, administration of atrazine (75 mg/kg) was associated with

increased resorptions, reduced fetal weights for both sexes, and increases in delayed ossification. In an unacceptable 3-generation reproduction study, no adverse reproductive effects were seen up to 1000 ppm, the highest dose tested.

3. Metabolism:

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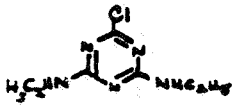
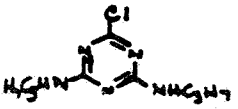
When a single oral dose of ^{14}C-atrazine was given to Long-Evans rats, 52-57% was excreted in the urine and 12-15% in the feces within 48 hours. Less than 0.1% was excreted in expired CO_2. The highest tissue residues were found in the liver and kidney. No metabolite identification was done in this study.

4. Structure Activity Relationship:

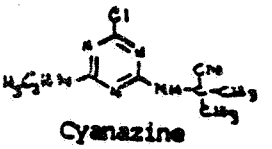
Atrazine is structurally related to simazine, cyanazine, propazine, and terbutryn, the structures of which are shown below.



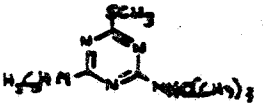
Simazine



Propazine



Cyanazine



Terbutryn

a. Simazine

Simazine is rapidly metabolized in the rat. Eighty-six percent of the labelled compound is excreted within 14 hours in the urine and feces. Oncogenicity studies are currently underway.

b. Cyanazine

In rats, 89% of labelled cyanazine is eliminated within 4 days, 42% in urine and 47% in feces. The major metabolic pathways are dechlorination and deethylation. Cyanazine did not produce chromosomal aberrations in bone marrow of mice and did not appear to be oncogenic to CD mice. Adequate oncogenicity studies the rat are not available; however, a new study in the rat is presently being conducted.

c. Propazine

Forty-two percent of ^{14}C-propazine was eliminated in the urine and 28% in the feces. Mostly unchanged propazine was found in the feces. Hydroxypropazine was identified both in urine and feces.

Propazine has been found to be positive for mutagenicity in V79 Chinese hamster cells both with and without metabolic activation. However, the response was weaker in the presence of metabolic activation. It was negative in a nucleus anomaly assay and in a DNA repair assay in rat hepatocytes.

Propazine was negative for oncogenicity in the CD-1 mouse but caused a statistically significant increase in mammary gland tumors in female CD rats. Propazine has recently been presented to the Toxicology Branch Peer Review Committee for classification of oncogenic potential and has been classified as a category C oncogen.

d. Terbutryn

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Eighty-five percent of ring-labelled ^{14}C-terbutryn is excreted within 72 hours in the urine (39%) and feces (46%) of rats. The major metabolic pathways are desulfuration, N-deethylation and S-demethylation.

Terbutryn is not mutagenic in the Ames Salmonella assay and the micronucleus assay and does not cause chromosomal aberrations in vivo in hamsters.

Terbutryn is negative for oncogenicity in the CD-1 mouse. When administered in the diet to female Charles River CD rats, terbutryn induced a statistically significant increase in combined mammary gland adenomas and adenocarcinomas and in combined hepatocellular adenomas and carcinomas. In males, terbutryn induced an increase in combined thyroid follicular cell adenomas and carcinomas and in testicular interstitial cell adenomas. The Toxicology Branch Peer Review Committee has classified terbutryn as a category C oncogen.

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Atrazine to be of importance in a weight of the evidence determination of oncogenic potential.

1. Administration of Atrazine to female Sprague-Dawley rats was associated with a statistically significant increase in mammary gland fibroadenomas at 1000 ppm, in mammary gland adenocarcinomas (including two carcinosarcomas at the HDT) at 70, 500, and 1000 ppm, and in total mammary gland tumor bearing animals at 1000 ppm. Each of these increases was associated with a statistically significant dose-related trend and was outside of the high end of the historical control range. In addition, there was evidence for decreased latency for mammary gland adenocarcinomas at the 12 month interim sacrifice.

2. A statistically significant increase in testicular interstitial cell tumors was seen in male Sprague-Dawley rats at 1000 ppm; however, this increase was within the historical control range.

3. In both males and females the highest dose tested exceeded the MTD based upon body weight gain decrement in males and increased mortality, liver necrosis, and bone marrow myeloid hyperplasia in the females. The MTD was reached in males and females at 500 ppm.

4. Testing for oncogenicity in the mouse is underway but has not been completed.

5. Atrazine was negative in three acceptable assays for mutagenicity.

6. Atrazine was not teratogenic in rats or rabbits and caused no reproductive toxicity in rats up to 1000 ppm.

7. Complete metabolism studies are not available; however, atrazine has been shown to be excreted mainly in the urine.

8. Atrazine is structurally related to simazine, cyanazine, propazine and terbutryn. Both propazine and terbutryn have been found to induce mammary tumors in rats and have been classified as category C oncogens by the Toxicology Branch Peer Review Committee. In addition, cyanazine, propazine and terbutryn have been found to be negative for oncogenicity in the CD-1 mouse.

3. Classification of Oncogenic Potential:

The Committee concluded that the data available for atrazine provided limited evidence for the oncogenicity of the chemical in rats. According to EPA Guidelines for Carcinogen Risk Assessment (CFR, September 24, 1986), the Committee classified Atrazine as a Category C oncogen (possible human carcinogen).

That is, administration of atrazine to Sprague-Dawley rats was associated with an increased incidence of mammary gland fibroadanomas and adenocarcinomas in female rats. The increase in testicular interstitial cell tumor seen at the high dose in male rats was not considered to be treatment-related by the Committee since the incidence was within the historical control range and was seen at a dosage level that exceeded the MTD. Atrazine has not shown any mutagenic activity in any assays available to the Committee; however, it is structurally related to propazine and terbutryn which induce mammary gland tumors in female rats and have been classified as category C oncogens.

The classification of atrazine as a possible human carcinogen was tentative pending receipt of an acceptable oncogenicity study in the mouse. The Committee concluded that a quantitative risk assessment should be performed due to (1) the induction of malignant mammary gland tumors and possible decreased latency for their appearance and (2) positive SAR data for mammary gland tumors.

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2. **Second Peer Review of Atrazine (8/1/88)**

FILE COPY

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY 006937
WASHINGTON, D.C. 20460

AUG 1 1988

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

Subject: Second Peer Review of Atrazine

From: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 7/7/88
Section Head, Section VI
Toxicology Branch/HED (TS-769C)

To: Robert Taylor/Clare Grubbs
Product Manager #25
Registration Division (TS-767C)

and

Jude Andreasen
Special Review Branch
Registration Division (TS-767C)

The Toxicology Branch Peer Review Committee met on June 6, 1988 to discuss and reevaluate the weight of the evidence on the oncogenic potential of atrazine in light of the results of a recently submitted oncogenicity study in the mouse.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with peer review unless otherwise stated).

Theodore M. Farber	Theodore M. Farber
William Burnam	William F Burnam
Reto Engler	Reto Engler
John A. Quest	John A. Quest
Esther Rinde	Esther Rinde
Judith W. Hauswirth	Judith W. Hauswirth
Lynnard Slaughter	L. J. Slaughter
Kerry Dearfield	Kerry Dearfield
Richard Levy	

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Robert Beliles

2. Scientific Reviewers: (Non-committee members responsible for presentation of data; signature indicates technical accuracy of panel report.)

Sanford Bigelow

3. Peer Review Committee Members in Absentia: (Committee members who were not able to attend the discussion; signatures indicated concurrence with the overall conclusions of the Committee.)

Anne Barton

Richard Hill

Diane Beal

Marion Copley

B. Material Reviewed:

The material available for review consisted of a package prepared by Dr. Hauswirth containing a data evaluation report of a mouse oncogenicity study, a Draft peer review report of the first atrazine meeting of September 10, 1987, and a review of the available mutagenicity data on atrazine (Dr. K. Dearfield, memorandum dated April 26, 1988).

C. Background Information:

Atrazine was tentatively classified as a category C oncogen by the Toxicology Branch Peer Review Committee on September 10, 1987 based upon the results of a chronic toxicity/oncogenicity study in the Sprague-Dawley rat. According to the report:

> ....administration of atrazine to Sprague-Dawley rats was associated with an increased incidence of mammary gland fibroadenomas and adenocarcinomas in female rats. The increase in testicular interstitial cell tumors seen at the high dose in male rats was not considered to be treatment-related by the Committee since the incidence was within the historical control range and was seen at a dosage level that exceeded the MTD. Atrazine has not shown any mutagenic activity in any assays available to the Committee; however, it is structurally related to propazine and terbutryn which induce mammary gland tumors in female rats and have been classified as category C oncogens.

D. Evaluation of Mouse Oncogenicity Study:

Ref.: Atrazine - technical: 91-week oral carcinogenicity study in mice. J.R. Hazelette and J. D. Green. Conducted by Divison of Toxicology

Ciba-Geigy Corp., Summit, NJ. Study No.: 842120. MRID No. 404313-02.

CD-1 [Crl: Cdl (ICR) BR] mice were placed on diets containing 0, 10, 300, 1500 and 3000 ppm atrazine. The number of mice in each group was as follows: 60 for the control, 300, 1500, and 3000 ppm female groups, 59 for the 10 ppm female group, 60 for the 10, 300 and 1500 ppm male groups, 59 for the male control group and 58 for the male 3000 ppm group.

This study has been classified as a Core guideline study with a NOEL of 300 ppm (45 mg/kg/day) and a LEL of 1500 ppm (225 mg/kg/day) based upon decreases of 23.5% and 11% in mean body weight gain found at 91 weeks in male and female mice, respectively and an increased incidence of cardiac thrombi found in female mice. Based upon the depression in body weight gain seen in male and female mice at 1500 and 3000 ppm and increased mortality of female mice at 3000 ppm, adequate dosage levels were tested to determine the oncogenic potential of atrazine in the mouse. No oncogenic effects were noted at any level that could be attributed to atrazine.

E. Additional Information:

The registrant submitted a position paper on the available mutagenicity data on atrazine, including studies conducted by the registrant and those found in the open literaure. This paper was written by Dr. D. Brusick and was reviewed by Dr. K. Dearfield in Toxicology Branch.

Dr. Dearfield concluded:

> It appears from a review of the many atrazine studies available to OPP from submissions and through the published literature that atrazine does not induce genotoxic activity in in vitro studies ± mammalian activation systems. However, there does appear to be genotoxic potential by atrazine as revealed in in vivo studies.

He noted that further mutagenicity testing on atrazine is required to satisfy the guideline requirements in this area and that atrazine is a plant activated promutagen suggesting that testing of isolated plant metabolites be conducted. Dr. Hauswirth noted that the registrant has informed her that mutagenicity testing has been conducted on one plant metabolite and that they were about to submit this data.

F. Classification of Oncogenic Potential:

The Committee concluded that the new data presented on atrazine did not alter their conclusion that atrazine was a category C oncogen and that a quantification of risk should be performed as outlined in their June 6, 1988 report of the first peer review meeting on atrazine. The oncogenicity study in the CD-1 mouse was negative for oncogenicity and the review of the mutagenicity data base on atrazine did not provide information that would change this categorization.

3. Third Peer Review of Atrazine (11/22/88)

006937

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Third Peer Review of Atrazine - Reevaluation Following the September 7, 1988 Scientific Advisory Panel Review

From: Marion P. Copley, D.V.M.
Acting Section Head, Section 2
Toxicology Branch I (IRS), HED (TS-769C)

To: Robert Taylor (PM 25)
Registration Division (TS-767C)

and

Jude Andreasen (TS-767C)
Special Review
Special Review and Reregistration Division

The Peer Review Committee met on September 29, 1988 to examine the issues raised by the Scientific Advisory Panel (SAP) with respect to the classification of the carcinogenicity of Atrazine.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated).

Theodore Farber — Theodore M. Farber
William Burnam — Wm E Burnam
Reto Engler — Reto Engler
Judith Hauswirth — Judith W. Hauswirth
Marsha van Gemert — Marsha van Gemert
Marion Copley — Marion Copley 10/7/88
Kerry Dearfield — Kerry Dearfield
Esther Rinde — Esther Rinde
John Quest — John A. Quest

Lynnard Slaughter

Robert Belile

2. Reviewers: (Non-committee members responsible for data presentation, signatures indicate technical accuracy of panel report).

Marion Copley (Reviewer)

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion: signatures indicate concurrence with the overall conclusions of the Committee.)

Richard Hill

Richard Levy

Diane Baal

B. Material Reviewed:

The SAP response memorandum from the September 7, 1988 meeting was reviewed by the Committee (see attachment 1).

C. Considerations:

The Panel agreed with the Committee's overall assessment of the weight of the evidence of Atrazine, classifying it as a category C oncogen. Although "the Panel believes that mammary tumors in Sprague-Dawley rats should be considered as a biologically significant endpoint", they did not agree that a quantification of risk should be performed, since 1) the tumor site of concern was the mammary gland in Sprague-Dawley rats and 2) "the issue was further complicated by the influence of secondary factors such as endocrine imbalance at high, but not low doses".

Issues discussed:

Quantification of Risk:

The Committee originally felt (Peer Review Memorandum dated August 1, 1988) that a quantitative estimation of the oncogenic potential should be performed because of the occurrence of primarily malignant mammary tumors at doses that had few signs of toxicity.

Upon reevaluation of the data and considering the comments of the SAP, the Peer Review Committee determined that the data for Atrazine are not appropriate for quantitative risk assessment

(using the Weibull model). This was because the tumors occurred only in one sex (female), species (rat) and strain (Sprague-Dawley). Mammary tumors occur at a variable rate, with a high background incidence in this strain of rat. Additional mutagenicity data have lessened the concern for this endpoint, therefore genotoxicity could not be used to support quantification. There appears to be, although not yet substantiated by data, a hormonal mechanism.

According to preliminary data submitted by the registrant, there appears to be a hormonal mechanism involved in the induction of mammary tumors by Atrazine. Information contained in HED files on other s-triazines, structurally similar to Atrazine, indicates that they induce tumors only in hormonally sensitive tissues. Additional information discussed at the SAP meeting, but not yet substantiated by actual data, indicates that female rats produced in the first or second generation of an Atrazine reproduction study, and continued on diets containing Atrazine for two years, did not have mammary gland tumors at a higher rate than the corresponding controls. This would also raise the question of hormonal influence in the production of mammary gland tumors by Atrazine.

In addition, there are sketchy data, generated by the registrant, indicating that the Sprague-Dawley rat may metabolize Atrazine differently than the Fischer rat and humans.

Interim Alternative to the Above Quantification of Risk:

The Committee members felt that since the mammary tumors were considered a toxicologic concern, the RfD, as used by Office of Pesticide Program (OPP) was not adequate, therefore they discussed alternative methods to account for the oncogenic potential. Until further information is evaluated, see below, the following was agreed upon by the Committee: Use the LHA (lifetime health advisory) level set by the Office of Drinking Water (ODW) for their Health Advisory for Atrazine (dated August 1988, attachment 2) rather than either the Q_1^* or the RfD. The ODW method differs from the current OPP policy for determining allowable dietary residues by including an additional uncertainty factor of 10 when calculating the lifetime drinking water health advisories (LHA), which are otherwise based on the Reference Dose (RfD), to account for possible carcinogenicity. The current RfD of 0.005 mg/kg/day, is based on a chronic dog NOEL of 0.48 mg/kg/day (cardiac effects). The value, after accounting for the additional uncertainty factor, would be 0.0005 mg/kg/day.

It should be noted that, although the Committee did not endorse a threshold mechanism, rat mammary tumors were significantly increased at 3.5 mg/kg/day (70 ppm) and above, not

at 0.5 mg/kg/day (10 ppm). In addition, in a Sprague-Dawley rat reproductive study, the NOEL for reproductive effects was also 0.5 mg/kg/day (10 ppm). Decreased pup weight was observed primarily at weaning with the next higher dose (50 ppm or 2.5 mg/kg/day). However, it could not be determined whether this effect was due to a maternal effect such as decreased milk production or direct pup toxicity from Atrazine.

New Data Needed to Reevaluate the Appropriate Risk Characterization Model

The listed studies are currently ongoing, or are in the planning stages at Ciba-Geigy. Preliminary results were pivotal in the above deliberations of the Peer Review Committee. Data provided by the completed studies are necessary for the Committee to determine the most appropriate method, if any, for quantifying the risk due to atrazine:

1) Hormone/receptor effects of Atrazine, including serum estrogen and other endocrine levels (including prolactin if possible);

2) Comparison of oncogenic potential between Fischer and Sprague-Dawley female rats;

3) Comparative metabolism between Fischer and Sprague-Dawley female rats, and between other mammalian species (rat hepatocytes).

D. Conclusions:

The Committee concluded that:

1) Atrazine should be classified as a category C oncogen.

2) The data were not convincing enough for quantitative risk characterization using the Weibull model.

3) The tumor response was of sufficient concern that the RfD should not become the default position for expressing long term risk levels for Atrazine.

Therefore, it was concluded that, until additional data (see above) are submitted to elucidate the most appropriate method of risk characterization, there should be an additional uncertainty factor of 10 added to the RfD to account for the oncogenic potential when determining allowable exposures to this compound.

The Committee strongly recommends that the registrant continue to generate data supporting a hormonal mechanism and

submit it to the Agency in a timely manner. The Committee also looks favorably upon the Registrant's decision to conduct an oncogenicity study in the Fischer rat. This information is required for the Committee to determine the most appropriate alternate method of risk determination.

COPLEY\PC6\ATRAZINE\PR3.RSP, 10/7/88

Attachment 1

006937

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review Classification of Atrazine as a Class C Oncogen

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The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed review of a set of scientific issues being considered by the Environmental Protection Agency's peer review classification of atrazine as a Class C oncogen. The review was conducted in an open meeting held in Arlington, Virginia, on September 7, 1988. All Panel members, except Dr. Thomas W. Clarkson, were present for the review.

Public notice of the meeting was published in the Federal Register on Monday, July 25, 1988.

Oral statements were received from staff of the Environmental Protection Agency and from Dr. James Stephens of Ciba-Geigy and Dr. Robert Squire of Johns Hopkins University representing Ciba-Geigy.

In consideration of all matters brought out during the meeting and careful review of all documents presented by the Agency, the Panel unanimously submits the following report.

REPORT OF PANEL RECOMMENDATIONS

Atrazine

The Agency requested the Panel to focus its attention upon a scientific issue relating to the Peer Review of atrazine. There follows the issues and the Panel's response to the issues:

Issue:

Atrazine was classified by the Toxicology Branch Peer Review Committee as C (Possible Human Carcinogen), based on: 1) increased incidence of tumors in one sex (primarily malignant tumors in females); 2) a possible mutagenicity concern and 3) a structure activity relationship with agents demonstrated to produce mammary tumors. The tumors associated with atrazine included mammary fibroadenoma/adenocarcinomas and adenocarcinoma in female rats.

1. The Agency requests any comments the Panel may wish to make regarding the biological significance of the mammary tumors in Sprague-Dawley rat.

Panel Response:

The Panel believes that mammary tumors in Sprague-Dawley rats should be considered as a biologically significant endpoint. As such, one relies not only on statistics to determine whether or not an effect is compound related, but also biological plausibility. The variability of this endpoint and its potential for secondary hormonal influence make this an important issue.

ISSUE:

2. Does the Panel have any specific comments regarding our overall assessment of the weight of evidence and classification of this chemical in accordance with the Agency's Guidelines for Carcinogen Risk Assessment.

Panel Response:

The Panel agrees with the Agency's classification of atrazine as a category C oncogen. We are, however, concerned about performing quantitative risk assessment (QRA) on the mammary tumor data. The Sprague-Dawley rat is clearly different from humans in sensitivity, contrary to an inherent assumption in QRA. The issue is further complicated by the influence of secondary factors such as endocrine imbalance at high, but not low doses. Therefore, the Panel recommends that QRA not be done on atrazine.

FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

Stephen L. Johnson
Executive Secretary
FIFRA Scientific Advisory Panel

Date: 9-14-87

Attachment 2

ATRAZINE

Health Advisory
Office of Drinking Water
U.S. Environmental Protection Agency

006937

I. INTRODUCTION

The Health Advisory (HA) Program, sponsored by the Office of Drinking Water (ODW), provides information on the health effects, analytical methodology and treatment technology that would be useful in dealing with the contamination of drinking water. Health Advisories describe nonregulatory concentrations of drinking water contaminants at which adverse health effects would not be anticipated to occur over specific exposure durations. Health Advisories contain a margin of safety to protect sensitive members of the population.

Health Advisories serve as informal technical guidance to assist Federal, State and local officials responsible for protecting public health when emergency spills or contamination situations occur. They are not to be construed as legally enforceable Federal standards. The HAs are subject to change as new information becomes available.

Health Advisories are developed for one-day, ten-day, longer-term (approximately 7 years, or 10% of an individual's lifetime) and lifetime exposures based on data describing noncarcinogenic end points of toxicity. For those substances that are known or probable human carcinogens, according to the Agency classification scheme (Group A or B), Lifetime HAs are not recommended. The chemical concentration values for Group A or B carcinogens are correlated with carcinogenic risk estimates by employing a cancer potency (unit risk) value together with assumptions for lifetime exposure and the consumption of drinking water. The cancer unit risk is usually derived from the linear multistage model with 95% upper confidence limits. This provides a low-dose estimate of cancer risk to humans that is considered unlikely to pose a carcinogenic risk in excess of the stated values. Excess cancer risk estimates may also be calculated using the one-hit, Weibull, logit or probit models. There is no current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than another. Because each model is based on differing assumptions, the estimates that are derived can differ by several orders of magnitude.

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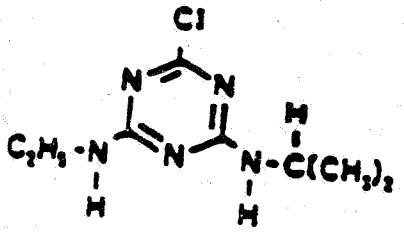
II. GENERAL INFORMATION AND PROPERTIES

CAS No. 1912-24-9

Structural Formula



2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine

Synonyms

- AAtrex; Atranex; Crisatrina; Crisazine; Farmco Atrazine; Griffex; Shell Atrazine Herbicide; Vectal SC; Gesaprim; Primatol (Meister, 1987).

Uses

- Atrazine over the past 30 years has been the most heavily used herbicide in the U.S. It is used for nonselective weed control on industrial or noncropped land and selective weed control in corn, sorghum, sugar cane, pineapple and certain other plants (Meister, 1987).

Properties (Meister, 1987; Windholz, 1976)

Chemical Formula	$C_8H_{14}ClN_5$
Molecular Weight	215.72
Physical State	White, ordorless, crystalline solid
Boiling Point (25 mm Hg)	--
Melting Point	175 to 177°C
Density (20°)	1.187
Vapor Pressure (20°C)	3.0×10^{-7} mm Hg
Water Solubility (22°C)	70 mg/L
Log Octanol/Water Partition Coefficient	2.33 to 2.71
Taste Threshold	--
Odor Threshold	--
Conversion Factor	--

Occurrence

- In a monitoring study of Mississippi River water, atrazine residues were found at a maximum level of 17 ppb; residues were detected throughout the year, with the highest concentrations found in June or July (Newby and Tweedy, 1976).

- Atrazine has been found in 4,123 of 10,942 surface water samples analyzed and in 143 of 3,208 ground water samples (STORET, 1988).

Samples were collected at 1,659 surface water locations and 2,510 ground water locations. The 85th percentile of all non-zero samples was 2.3 ug/L in surface water and 1.9 ug/L in ground water sources. The maximum concentration found in surface water was 2,300 ug/L and in ground water it was 700 ug/L. Atrazine was foound in surface water of 31 States and in ground water in 13 States. This information is provided to give a general impression of the occurrence of this chemical in ground and surface waters as reported in the STORET database. The individual data points retrieved were used as they came from STORET and have not been confirmed as to their validity. STORET data is often not valid when individual numbers are used out of the context of the entire sampling regime, as they are here. Therefore, this information can only be used to form an impression of the intensity and location of sampling for a particular chemical.

- Atrazine has been found also in ground water in Pennsylvania, Iowa, Nebraska, Wisconsin and Maryland; typical positives were 0.3 to 3 ppb (Cohen et al., 1986).

Environmental Fate

- An aerobic soil metabolism study in Lakeland sandy loam, Hagerstown silty clay loam, and Wehadkee silt loam soils showed conversion of atrazine to hydroxyatrazine, after 8 weeks, to be 38, 40 and 47% of the amount applied, respectively, (Harris, 1967). Two additional degradates, deisopropylated atrazine and deethylated atrazine, were identified in a sandy loam study (Beynon et al., 1972).

- Hurle and Kibler (1976) studied the effect of water-holding capacity on the rate of degradation and found a half-life for atrazine of more than 125 days, 37 days and 36 days in sandy soil held at 4%, 35% and 70% water-holding capacity, respectively.

- In Oakley sandy loam and Micollet clay loam, atrazine had a half-life of 101 and 167 days (Warnock and Leary, 1978).

- Carbon dioxide production was generally slow in several anaerobic soils: sandy loam, clay loam, loamy sand and silt loam (Wolf and Martin, 1975; Goswami and Green, 1971; Lavy et al., 1973).

- ^{14}C-Atrazine was stable in aerobic ground water samples incubated for 15 months at 10 or 25°C in the dark (Weidner, 1974).

- Atrazine is moderately to highly mobile in soils ranging in texture from clay to gravelly sand as determined by soil thin layer chromatography (TLC), column leaching, and adsorption/desorption batch equilibrium studies. Atrazine on soil TLC plates was intermediately mobile in loam, sandy clay loam, clay loam, silt loam, silty clay loam, and silty clay soils, and was mobile in sandy loam soils. Hydroxyatrazine showed a low mobility in sandy loam and silty clay loam soils (Helling, 1971).

- Soil adsorption coefficients for atrazine in a variety of soils were: sandy loam (0.6), gravelly sand (1.8), silty clay (5.6), clay loam (7.9), sandy loam (8.7), silty clay loam (11.6), and peat (more than 21) (Weidner, 1974; Lavy 1974; Talbert and Fletchall, 1965).

- Soil column studies indicated atrazine was mobile in sand, fine sandy loam, silt loam and loam; intermediately mobile in sand, silty clay loam and sandy loam; low to intermediately mobile in clay loam (Weidner, 1974; Lavy, 1974; Ivey and Andrews, 1964; Ivey and Andrews, 1965).

- In a Mississippi field study, atrazine in silt loam soil had a half-life of less than 30 days (Portnoy, 1978). In a loam to silt loam soil in Minnesota, atrazine phytotoxic residues persisted for more than 1 year and were detected in the maximum-depth samples (30 to 42 inches) (Darwent and Behrens, 1968). In Nebraska, phytotoxic residues persisted in silty clay loam and loam soils 16 months after application of atrazine; they were found at depths of 12 to 24 inches. But atrazine phytotoxic residues had a half-life of about 20 days in Alabama fine sandy loam soil, although leaching may partially account for this value (Buchanan and Hiltbold, 1973).

- Under aquatic field conditions, dissipation of atrazine was due to leaching and to dilution by irrigation water, with residues persisting for 3 years in soil on the sides and bottoms of irrigation ditches, to the maximum depth sampled, 67.5 to 90 cm (Smith et al., 1975).

- Ciba-Geigy (1988) recently submitted comments on the atrazine Health Advisory. These comments included a summary of the results of its studies on the environmental fate of atrazine. This summary indicated that laboratory degradation studies showed that atrazine is relatively stable in the aquatic media under environmental pH conditions and indicated that atrazine degraded in soil by photolysis and microbial processes. The products of degradation are dealkylated metabolites, hydroxyatrazine and nonextractable (bound) residues. Atrazine and the dealkylated metabolites are relatively mobile whereas hydroxyatrazine is immobile.

- Ciba-Geigy (1988) also indicated that field dissipation studies conducted in California, Minnesota and Tennessee show no leaching of atrazine and metabolites below 6 to 12 inches of soil. The half-lives of atrazine in soil ranged between 20 to 101 days, except in Minnesota where degradation was slow. A forestry degradation study conducted in Oregon showed no adverse effects on either terrestial or aquatic environments. Also, Bioconcentration studies have shown low potential for bioaccumulation with a range of 15 to 77X.

II. PHARMACOKINETICS

Absorption

- Atrazine appears to be readily absorbed from the gastrointestinal tract of animals. Bakke et al. (1972) administered single 0.53-mg doses of ^{14}C-ring-labeled atrazine to rats by gavage. Total fecal

excretion after 72 hours was 20.3% of the administered dose; the remainder was excreted in urine (65.5%) or retained in tissues (15.8%). This indicates that at least 80% of the dose was absorbed.

Distribution

- Bakke et al. (1972) administered single 0.53-mg doses of ^{14}C-ring-labeled atrazine to rats by gavage. Liver, kidney and lung contained the largest amounts of radioactivity, while fat and muscle had lower residues than the other tissues examined.

- In a metabolism study by Ciba-Geigy (1983a), the radioactivity of ^{14}C-atrazine dermally applied to Harlan Sprague-Dawley rats at 0.25 mg/kg was distributed to a minor extent to body tissues. The highest levels were measured in liver and muscle at all time points examined; 2.1% of the applied dose was in muscle and 0.5% in liver at 8 hours.

- Khan and Foster (1976) observed that in chickens the hydroxy metabolites of atrazine accumulate in the liver, kidney, heart and lung. Residues of both 2-chloro and 2-hydroxy moieties were found in chicken gizzard, intestine, leg muscle, breast muscle and abdominal fat.

Metabolism

- The principal reactions involved in the metabolism of atrazine are dealkylation at the C-4 and C-6 positions of the molecule. There is also some evidence of dechlorination at the C-2 position. These data were reported by several researchers as demonstrated below.

- Bakke et al. (1972) administered single 0.53-mg doses of ^{14}C-ring-labeled atrazine to rats by gavage. Less than 0.1% of the label appeared in carbon dioxide in expired air. Most of the radioactivity was recovered in the urine (65.5% in 72 hours), including at least 19 radioactive compounds. More than 80% of the urinary radioactivity was identified as 2-hydroxyatrazine and its two mono-N-dealkylated metabolites. None of the metabolites identified contained the 2-chloro moiety (which may have been removed via hydrolysis during the isolation technique or by a dechlorinating enzyme as suggested by the *in vitro* studies of Foster et al. (1979), who found evidence for a dechlorinase in chicken liver homogenates incubated with atrazine).

- Behme and Bar (1967) identified five urinary metabolites of atrazine in rats: the two monodealkylated metabolites of atrazine, their carboxy acid derivatives and the fully dealkylated derivative. All of these metabolites contained the 2-chloro group. The *in vitro* studies of Dauterman and Muecke (1974) also found no evidence for dechlorination of atrazine in the presence of rat liver homogenates.

- Similarly, Bradway and Moseman (1982) administered atrazine (50, 5, 0.5 or 0.005 mg/day) for 3 days to male Charles River rats and observed that the fully dealkylated derivative (2-chloro-4,6-diamino-s-triazine) was the major urinary metabolite, with lesser amounts of the two mono-N-dealkylated derivatives.

- Erickson et al. (1979) dosed Pittman-Moore miniature pigs by gavage with 0.1 g of atrazine (80W). The major compounds identified in the urine were the parent compound (atrazine) and deethylated atrazine (which contains the 2-chloro substituent).

- Hauswirth (1988) indicated that the rat metabolism studies taken together are sufficient to show that in the female rat dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways. Oxidation of the alkyl substituents appears to be a minor and secondary metabolic route. The total body half-life is approximately one and one-half days. Atrazine and/or its metabolites appear to bind to red blood cells. Other tissue accumulation does not appear to occur.

Excretion

- Urine appears to be the principal route of atrazine excretion in animals. Following the administration of 0.5 mg doses of ^{14}C-ring-labeled atrazine by gavage to rats, Bakke et al. (1972) reported that in 72 hours most of the radioactivity (65.5%) was excreted in the urine, 20.3% was excreted in the feces, and less than 0.1% appeared as carbon dioxide in expired air. About 85 to 95% of the urinary radioactivity appeared within the first 24 hours after dosing, indicating rapid clearance.

- Dauterman and Muecke (1974) have reported that atrazine metabolites are conjugated with glutathione to yield a mercapturic acid in the urine. The studies of Foster et al. (1979) in chicken liver homogenates also indicate that atrazine metabolism involves glutathione.

- Ciba-Geigy (1983b) studied the excretion rate of ^{14}C-atrazine from Harlan Sprague-Dawley rats dermally dosed with atrazine dissolved in tetrahydrofuran at levels of 0.025, 0.25, 2.5 or 5 mg/kg. Urine and feces were collected from all animals at 24-hour intervals for 144 hours. Results indicated that atrazine was readily absorbed, and within 48 hours most of the absorbed dose was excreted, mainly in the urine and to a lesser extent in the feces. Cumulative excretion in urine and feces appeared to be directly proportional to the administered dose, ranging from 52% at the lowest dose to 80% at the highest dose.

IV. HEALTH EFFECTS

Humans

Short-term Exposure

- A case of severe contact dermatitis was reported by Schlicher and Beat (1972) in a 40-year-old farm worker exposed to atrazine formulation. The clinical signs were red, swollen and blistered hands with hemorrhagic bullae between the fingers. Although it is noted that the exposure of this patient may have been inclusive to exposure to other chemicals in addition to atrazine, it is also noted that atrazine is a skin irritant in animal studies.

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Long-term Exposure

- Yoder et al. (1973) examined chromosomes in lymphocyte cultures taken from agricultural workers exposed to herbicides including atrazine. There were more chromosomal aberrations in the workers during mid-season exposure to herbicides than during the off-season (no spraying). These aberrations included a four-fold increase in chromatid gaps and a 25-fold increase in chromatid breaks. During the off-season, the mean number of gaps and breaks was lower in this group than in controls who were in occupations unlikely to involve herbicide exposure. This observation led the authors to speculate that there is enhanced chromosomal repair during this period of time resulting in compensatory protection. However, these data may not be representative of the effect of atrazine since the exposed workers were also exposed to other herbicides.

Animals

Short-term Exposure

- Acute oral LD_{50} values of 3,000 mg/kg in rats and 1,750 mg/kg in mice have been reported for technical atrazine by Rashmurin (1974); the purity of the test compound was not specified.

- Acute oral studies conducted by Ciba-Geigy (1988) with atrazine (97% a.i.) reflected the following LD_{50}s: 1,869 mg/kg in rats and >3,000 mg/kg in mice.

- Molnar (1971) reported that when atrazine was administered by gavage to rats at 3,000 mg/kg, 6% of the rats died within 6 hours, and 25% of those remaining died within 24 hours. The rats that died during the first day exhibited pulmonary edema with extensive hemorrhagic foci, cardiac dilation and microscopic hemorrhages in the liver and spleen. Rats that died during the second day had hemorrhagic broncho-pneumonia and dystrophic changes of the renal tubular mucosa. Rats sacrificed after 24 hours had cerebral edema and histochemical alterations in the lungs, liver and brain. It is noted that the dose used in this study was almost 2 x the LD_{50} (Ciba-Geigy, 1988).

- Gaines and Linder (1986) determined the oral LD_{50} for adult male and female rats to be 737 and 672 mg/kg respectively and 2,310 mg/kg for pups. It is, therefore, noted that young animals are more sensitive to atrazine than adults. This study also reflected that the dermal LD_{50} for adult rats was higher than 2,500 mg/kg.

- Palmer and Radeleff (1964) administered atrazine as a fluid dilution or in gelatin capsules to Dalaine sheep and dairy cattle (one animal per dosage group). Two doses of 250 mg/kg atrazine caused death in both sheep and cattle. Sixteen doses of 100 mg/kg were lethal to the one sheep tested. At necropsy, degeneration and discoloration of the adrenal glands and congestion in lungs, liver and kidneys were observed.

- Palmer and Radeleff (1969) orally administered atrazine 80W (analysis of test material not provided) by capsule or by drench to sheep at 5, 10, 25, 50, 100, 250 or 400 mg/kg/day and to cows at 10, 25, 50, 100 or 250 mg/kg/day. The number of animals in each dosage group was not stated, and the use of controls was not indicated. Observed effects included muscular spasms, stilted gait and stance and anorexia at all dose levels in sheep and at 25 mg/kg in cattle. Necropsy revealed epicardial petechiae (small hemorrhagic spots on the lining of the heart) and congestion of the kidneys, liver and lungs. Effects appeared to be dose related. A Lowest-Observed-Adverse-Effect Level (LOAEL) of 5 mg/kg/day in sheep and a No-Observed-Adverse-Effect Level (NOAEL) of 10 mg/kg/day in cows can be identified from this study.

- Bashmurin (1974) reported that oral administration of 100 mg/kg of atrazine to cats had a hypotensive effect, and that a similar dose in dogs was antidiuretic and decreased serum cholinesterase (ChE) activity. No other details of this study were reported. Atrazine is not an organophosphate (OP), therefore, its effect on ChE may not be similar to the mechanism of ChE inhibition by OPs.

Dermal/Ocular Effects

- In a primary dermal irritation test in rats, atrazine at 2,800 mg/kg produced erythema but no systemic effects (Gaheyotskiy et al., 1977).

- Ciba-Geigy (1988) indicated that the studies it performed reflected dermal sensitization in rats but not irritation in rabbits' eyes.

Long-term Exposure

- Hazelton Laboratories (1961) fed atrazine to male and female rats for 2 years at dietary levels of 0, 1, 10 or 100 ppm. Based on the dietary assumptions of Lehman (1959), these levels correspond to doses of approximately 0, 0.05, 0.50 or 5.0 mg/kg/day. After 65 weeks, the 1.0-ppm dose was increased to 1,000 ppm (50 mg/kg/day) for the remainder of the study. No treatment-related pathology was found at 26 weeks, at 52 weeks, at 2 years, or in animals that died and were necropsied during the study. Results of blood and urine analyses were unremarkable. Atrazine had no effects on the general appearance or behavior of the rats. A transient roughness of the coat and piloerection were observed in some animals after 20 weeks of treatment at the 10- and 100-ppm levels but not at 52 weeks. Body weight gains, food consumption and survival were similar in all groups for 18 months, but from 18 to 24 months there was high mortality due to infections (not attributed to atrazine) in all groups, including controls, which limits the usefulness of this study in determining a NOAEL for the chronic toxicity of atrazine.

- In a 2-year study by Woodard Research Corporation (1964), atrazine (80W formulation) was fed to male and female beagle dogs for 105 weeks at dietary levels of 0, 15, 150 or 1,500 ppm. Based on the dietary assumptions of Lehman (1959), these levels correspond to doses of 0, 0.35, 3.5 or 35 mg/kg/day. Survival rates, body weight

gain, food intake, behavior, appearance, hematologic findings, urinalyses, organ weights and histologic changes were noted. The 15-ppm dosage (0.35 mg/kg/day) produced no toxicity, but the 150-ppm dosage (3.5 mg/kg/day) caused a decrease in food intake as well as increased heart and liver weight in females. In the group receiving 1,500 ppm (35 mg/kg/day) atrazine, there were decreases in food intake and body weight gain, an increase in adrenal weight, a decrease in hematocrit and occasional tremors or stiffness in the rear limbs. There were no differences among the different groups in the histology of the organs studied. Based on these results, a NOAEL of 0.35 mg/kg/day can be identified for atrazine.

- In a study by Ciba-Geigy (1987b) using technical atrazine (97% a.i.), six-month-old beagle dogs were assigned randomly to four dosage groups: 0, 15, 150 and 1,000 ppm. These doses correspond to actual average intake of 0, 0.48, 4.97 and 33.65/33.8 (male/female) mg/kg/day. Six animals/sex/group were assigned to the control and high dose groups and four animals/sex/group were assigned to the low- and mid-dose groups. One mid-dose male, one high-dose male and one high-dose female had to be sacrificed moribund during the study period. Decreased body weight gains and food consumption were noted at the high-dose level. Statistically significant ($p < 0.05$) reductions in erythroid parameters (red cell count, hemoglobin and hematocrit) in high-dose males were noted throughout the study as well as mild increases in platelet counts in both sexes. Slight decreases in total protein and albumin ($p < 0.05$) were noted in high-dose males as well as decreased calcium and chloride in males and increased sodium and glucose in females. Decrease in absolute heart weight were noted in females and increased relative liver weight in males of the high-dose group. The mid-dose females reflected an increase in the absolute heart weight and heart/brain weight ratios. The most significant effect of atrazine in this study was reflected in the high-dose animals of both sexes as discrete myocardial degeneration. Clinical signs associated with cardiac pathology such as ascites, cachexia, labored/shallow breathing and abnormal EKG were observed in the group as early as 17 weeks into the study. Gross pathology reflected severe dilation of the right atrium and occasionally of the left atrium. These findings were also noted histopathologically as degenerative atrial myocardium (atrophy and myolysis). In the mid-dose group, two males and one female appeared to be affected with the cardiac syndrome but to a much lesser degree in the intensity of the noted responses. Therefore, the LOAEL in this study is 4.97 mg/kg/day and the NOAEL is 0.48 mg/kg/day.

- A two year chronic feeding/oncogenicity study (Ciba-Geigy, 1986) was recently evaluated by the Agency. In this study, technical atrazine (98.9% a.i.) was fed to 37 to 38 days-old Sprague-Dawley rats. The dosage levels used were 0, 10, 70, 500 or 1,000 ppm, equivalent to 0, 0.5, 3.5, 25 or 50 mg/kg/day (using Lehman's conversion factor, 1959). Twenty rats per sex per group were used to measure blood parameters and clinical chemistries and urinalysis. Fifty rats per sex per goup were maintained on the treated and control diets for 24 months. An additional 10 rats per sex were placed on control and high dose (1,000 ppm) diets for a twelve month interim sacrifice and

another 10 per sex (control and high dose, 1,000 ppm) for a 13 month sacrifice (the 1,000 ppm group was placed on control diet for one month prior to sacrifice). The total number of animals/sex in the control and HDT groups was 90 and 70 for the 10, 70 and 500 ppm groups. Histopathology was performed on all animals. At the mid- and high-dose, there was a decrease in mean body weights of males and females. Survival was decreased in high-dose females but increased in high-dose males. There were decreases in organ-to-body weight ratios in high-dose animals, which were probably the result of body weight decreases. Hyperplastic changes in high-dose males (mammary gland, bladder and prostate) and females (myeloid tissue of bone marrow and transitional epithelium of the kidney) were of questionable toxicologic importance. There was an increase in retinal degeneration and in centrolobular necrosis of the liver in high-dose females and an increase in degeneration of the rectus femoris muscle in high-dose males and females when compared to controls. Based on decreased body weight gain, the LOAEL for non-oncogenic activities in both sexes is 25 mg/kg/day and the NOAEL is 3.5 mg/kg/day. However, oncogenic activities were noted at 3.5 mg/kg/day (70 ppm) and above as reflected in the increased incidence of mammary gland tumors in females.

- A recent 91-week oral feeding/oncogenicity study in mice by Ciba-Geigy (1987c) has been evaluated by the Agency. In this study, atrazine (97% ai.) was fed to five-weeks-old CD-1 strain of mice, weighing 21.0/26.8 grams (female/male). The mice were randomly assigned to five experimental groups of approximately 60 animals/sex/group. The dosage tested were 0, 10, 300, 1,500 and 3,000 ppm; these dosages correspond to actual mean daily intake of 1.4, 38.4, 194.0 and 385.7 mg/kg/day for males, and 1.6, 47.9, 246.9 and 482.7 mg/kg/day for females. This study shows that there are dose-related effects at 1,500 ppm or 3,000 ppm atrazine: an increase in cardiac thrombi, a decrease in the mean body weight gain at 12 and 91 weeks during the study, and decreases in erythrocyte count, hematocrit and hemoglobin concentration. Cardiac thrombi contributed to the deaths of the group of mice that did not survive to terminal sacrifice. The LOAEL is set at 1,500 ppm based upon decreases of 23.5% and 11.0% in mean body weight gain found at 91 weeks in male and female mice, respectively. Also, an increase in the incidence of cardiac thrombi is found in female mice in the 1,500 ppm exposure group. None of the above effects are found at 300 ppm, thus the NOAEL is set at 300 ppm (corresponding to 38.4 mg/kg/day in males and 47.9 mg/kg/day for females).

Reproductive Effects

- A three-generation study on the effects of atrazine on reproduction in rats was conducted by Woodard Research Corporation (1965). Groups of 10 males and 20 females received atrazine (80W) at dietary levels of 0, 50 or 100 ppm. Based on the dietary assumptions that 1 ppm in the diet of rats is equivalent to 0.05 mg/kg/day (Lehman, 1959), these levels correspond to doses of approximately 0, 2.5 or 5 mg/kg/day. Two litters were produced per generation but parental animals were chosen from the second litter after weaning for each generation. Young rats were maintained on the test diets for approximately ten

weeks in each generation. The third generation pups were sacrificed after weaning. It is noted that the parental animals of the first generation were fed only half of the dietary atrazine levels for the first 3 weeks of exposure. There were no adverse effects of atrazine on reproduction observed during the course of the three-generation study. A NOAEL of 100 ppm (5 mg/kg/day) was identified for this study. However, the usefulness of this study is limited due to the alteration of the atrazine content of the diet during important maturation periods of the neonates.

- A recent two-generations study in rats by Ciba-Geigy (1987a) was conducted using the 97% ai. technical atrazine. Young rats, 47 to 48 days old were maintained on the control and test diets for 10 weeks before mating. The concentrations used were 0, 10, 50 and 500 ppm (equivalent to 0, 0.5, 2.5 and 25 mg/kg/day using Lehman conversion factor, 1959). Thirty animals/sex/group were used in each generation; one litter was produced per generation. The level tested had no effect on mortality in either generation. Body weight and body weight gains were significantly depressed ($p < 0.05$) at the highest dose; however, food consumption was also decreased at this high-dose level in parental males and females during the premating period and for the first generation females (F_1) on days 0 to 7 of gestation. No histopathological effects were noted nor other effects were noted during gross necropsy in either parental generation with the exception of increased testes relative weight in both generations at the high dose. In pups of both generation, significant reduction ($p < 0.05$) in body weight was noted; however, this effect was only dose-related in the second generation (F_2) at both the mid- and high-dose levels on postnatal day 21. Therefore, maternal toxicity NOAEL is 2.5 mg/kg/day; the reproductive LOAEL is 2.5 mg/kg/day (reduced pup weight in F_2 generation on postnatal day 21) and the NOAEL is 0.5 mg/kg/day.

Developmental Effects

- In the three-generation reproduction study in rats conducted by Woodard Research Corporation (1966) (described above), atrazine at dietary levels of 50 or 100 ppm (2.5 or 5 mg/kg/day) resulted in no observed histologic changes in the weanlings and no effects on fetal resorption. No malformations were observed, and weanling organ weights were similar in controls and atrazine-treated animals. Therefore, a NOAEL of 100 ppm (5 mg/kg/day) was also identified for developmental effects in this study. However, the usefulness of this study is limited due to an alteration of the atrazine content of the diet during important maturation periods of the neonates.

- Atrazine was administered orally to pregnant rats on gestation days 6 to 15 at 0, 100, 500 or 1,000 mg/kg (Ciba-Geigy, 1971). The two higher doses increased the number of embryonic and fetal deaths, decreased the mean weights of the fetuses and retarded the skeletal development. No teratogenic effects were observed. The highest dose (1,000 mg/kg) resulted in 23% maternal mortality and various toxic symptoms. The 100 mg/kg dose had no effect on either dams or embryos and is therefore the maternal and fetotoxic NOAEL in this study.

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- In a study by Ciba-Geigy (1984a), Charles River rats received atrazine (97%) by gavage on gestation days 6 to 15 at dose levels or 0, 10, 70, or 700 mg/kg/day. Excessive maternal mortality (21/27) was noted at 700 mg/kg/day, but no mortality was noted at the lower doses; also reduced weight gains and food consumption were noted at both 70 and 700 mg/kg/day. Developmental toxicity was also present at these dose levels. Fetal weights were severely reduced at 700 mg/kg/day; delays in skeletal development occurred at 70 mg/kg/ day, and a dose-related runting was noted at 10 mg/kg/day and above. The NOAEL for maternal toxicity appears to be 10 mg/kg/day, however, this is also the LOAEL for developmental effects.

- New Zealand white rabbits received atrazine (96%) by gavage on gestation days 7 through 19 at dose levels of 0, 1, 5 or 75 mg/kg/day (Ciba-Geigy, (1984b). Maternal toxicity, evidenced by decreased body weight gains and food consumption, was present in the mid- and high-dose groups. Developmental toxicity was demonstrated only at 75 mg/kg/day by an increased resorption rate, reduced fetal weights, and delays in ossification. No teratogenic effects were indicated. The NOAEL appears to be 1 mg/kg/day.

- Peters and Cook (1973) fed atrazine to pregnant rats (four/group) at levels of 0, 50, 100, 200, 300, 400, 500 or 1,000 ppm in the diet throughout gestation. Based on an assumed body weight of 300 g and a daily food consumption of 12 g (Arrington, 1972), these levels correspond to approximately 0, 2, 4, 8, 12, 16, 20 or 40 mg/kg/day. The number of pups per litter was similar in all groups, and there were no differences in weanling weights. This study identified a NOAEL of 40 mg/kg/day for developmental effects. In another phase of this study, the authors demonstrated that subcutaneous (sc) injections of 50, 100 or 200 mg/kg atrazine on gestation days 3, 6 and 9 had no effect on the litter size, while doses of ≥800 mg/kg were embryotoxic. Therefore, a NOAEL of 200 mg/kg by the sc route was identified for embryotoxicity.

Mutagenicity

- Loprieno et al. (1980) reported that single doses of atrazine (1,000 mg/kg or 2,000 mg/kg, route not specified) produced bone marrow chromosomal aberrations in the mouse. No other details of this study were provided.

- Murnik and Nash (1977) reported that feeding 0.01% atrazine to male Drosophila melanogaster larvae significantly increased the rate of both dominant and sex-linked recessive lethal mutations. They stated, however, that dominant lethal induction and genetic damage may not be directly related.

- Adler (1980) reviewed unpublished work on atrazine mutagenicity carried out by the Environmental Research Programme of the Commission of the European Communities. Mutagenic activity was not induced even when mammalian liver enzymes (S-9) were used; however, the use of plant microsomes produced positive results. Also, in in vivo studies

in mice, atrazine induced dominant lethal mutations and increased the frequency of chromatid breaks in bone marrow. Hence, the author suggested that activation of atrazine in mammals occurs independently of the liver, possibly in the acidic part of the stomach.

- As described previously, Yoder et al. (1973) studied chromosomal aberrations in the lymphocyte cultures of farm workers exposed to various pesticides including atrazine. During mid-season a 4-fold increase in chromatid gaps and a 25-fold increase in chromatid breaks was observed. During the off-season (no spraying), the number of gaps and breaks was lower than in controls, suggesting to the authors that there is enhanced chromosomal repair during the unexposed period.

- Recently, Spencer (1987) and Dearfield (1988) evaluated several *in vitro* and *in vivo* mutagenicity studies on atrazine that were recently submitted to the U.S. EPA by Ciba-Geigy. They noted that most of these studies were inadequate with the exception of the following three tests: a *Salmonella* assay; an *E. coli* reversion assay; and a Host-Mediated assay. The first two assays were negative for mutagenic effects; the results of the third assay were equivocal.

- Ciba-Geigy (1988) indicated that Brusick (1987) evaluated atrazine mutagenicity and that the weight-of-evidence analysis he used placed the chemical in a non-mutagenic status. The Agency (Dearfield, 1988) evaluated Brusick's analysis. It is noted that the use of the weight-of-evidence approach is not appropriate at the present time. The *in vivo* studies by Adler (1980) suggest a positive response. These findings have not been diminished by other atrazine studies. In addition, Dearfield (1988) indicated that the scheme used by Brusick in this analysis is flawed by the lack of calibration of the chemical test scores to an external standard and by the use of some studies that are considered inadequate by design to determine the mutagenic potential of atrazine.

Carcinogenicity

- Innes et al. (1969) investigated the tumorigenicity of 120 test compounds including atrazine in mice. Two F_1 hybrid stocks (C57BL/6 x Anf) F_1 and (C57BL/6 x AKR) F_1 were used. A dose of 21.5 mg/kg/day was administered by gavage to mice of both sexes from age 7 to 28 days. After weaning at 4 weeks, this dose level was maintained by feeding 82 ppm atrazine *ad libitum* in the diet for 18 months. The incidence of hepatomas, pulmonary tumors, lymphomas and total tumors in atrazine-treated mice was not significantly different from that in the negative controls.

- A two-year feeding/oncogenicity study in rats by Ciba-Geigy (1986) has been evaluated recently by the Agency. Atrazine (98.9% a.i.) was fed to 37 to 38 days-old Sprague-Dawley rats. The dosage levels used were 0, 10, 70, 500 or 1,000 ppm, equivalent to 0, 0.5, 3.5, 25 or 50 mg/kg/day (using Lehman's conversion factor, 1959). The total number of animals/sex in the control and HDT groups was 90; and 70 animals/sex/group for the 10, 70 and 500 ppm groups. Histopathology

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was performed on all animals. In females, atrazine was associated with a statistically significant increase in mammary gland fibroadenomas at 1,000 ppm. In mammary gland adenocarcinomas (including two carcinosarcomas at the HDT) at 70, 500 and 1,000 ppm, and in total mammary gland tumor bearing animals at 1,000 ppm. Each of these increases was associated with a statistically significant dose-related trend and was outside of the high end of the historical control range. In addition, U.S. EPA (1986a) indicated that there was evidence for decreased latency for mammary gland adenocarcinomas at the 12 month interim sacrifice that was already submitted by Ciba-Geigy in 1985. This study was also reported as positive in a briefing paper by Ciba-Geigy (1987).

- A recent 91-week oral feeding/oncogenicity study in mice by Ciba-Geigy (1987c) has been evaluated by the Agency. In this study, atrazine (97% ai.) was fed to five-weeks-old CD-1 mice weighing 21.0/26.8 grams (female/male). The mice were randomly assigned to five experimental groups of approximately 60 animals/sex/ group. The dosage tested were 0, 10, 300, 1,500 and 3,000 ppm; these dosages correspond to actual mean daily intake of 1.4, 38.4, 194.0 and 385.7 mg/kg/day for males, and 1.6, 47.9, 246.9 and 482.7 mg/kg/day for females. The following kinds of neoplasms were noted in this study: mammary adenocarcinomas, adrenal adenomas, pulmonary adenomas and malignant lymphomas. However, no dose-related or statistically significant increases were observed in the incidences of these neoplasms. Therefore, atrazine is not considered oncogenic in this strain of mice.

V. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Health Advisories (HAs) are generally determined for one-day, ten-day, longer-term (up to 7 years) and lifetime exposures if adequate data are available that identify a sensitive noncarcinogenic end point of toxicity. The HAs for noncarcinogenic toxicants are derived using the following formula:

$$HA = \frac{(\text{NOAEL or LOAEL}) \times (\text{BW})}{(\text{UF}) \times (\_\_\_ \text{ L/day})} = \_\_\_ \text{ mg/L } (\_\_\_ \text{ ug/L})$$

where:

NOAEL or LOAEL = No- or Lowest-Observed-Adverse-Effect Level in mg/kg bw/day.

BW = assumed body weight of a child (10 kg) or an adult (70 kg).

UF = uncertainty factor (10, 100, 1,000 or 10,000) in accordance with EPA or NAS/ODW guidelines.

___ L/day = assumed daily water consumption of a child (1 L/day) or an adult (2 L/day).

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One-day Health Advisory

No suitable information was found in the available literature for the determination of the One-day HA value for atrazine. It is, therefore, recommended that the Ten-day HA value calculated below for a 10-kg child of 0.1 mg/L (100 ug/L), be used at this time as a conservative estimate of the One-day HA value.

Ten-day Health Advisory

Two teratology studies by Ciba-Geigy, one in the rat (1984a) and one in the rabbit (1984b), were considered for the calculation of the Ten-day HA value. The rat study reflected a NOAEL of 10 mg/kg/day for maternal toxicity but this value was also the LOAEL for developmental toxicity while the rabbit study reflected NOAELs of 5 mg/kg/day for developmental toxicity and 1 mg/kg/day for maternal toxicity. Thus, the rabbit appears to be a more sensitive species than the rat for maternal toxicity, hence, the rabbit study with a NOAEL of 1 mg/kg/day is used in the calculations below.

The Ten-day HA for a 10 kg child is calculated below as follows:

$$\frac{(1\ \text{mg/kg/d}) \times (10\text{kg})}{(100) \times (1\ \text{L/day})} = 0.1\ \text{mgL}\ (100\ \text{ug/L})$$

where:

1 mg/kg/day = NOAEL, based on maternal toxicity evidenced by decreased body weight gain and food consumption.

10 kg = assumed body weight of a child.

100 = uncertainty factor, chosen in accordance with EPA or ODW/HAS guidelines for use with a NOAEL from an animal study.

1 L/day = assumed daily consumption for a child.

Longer-term Health Advisory

No suitable information was found in the available literature for the determination of the longer-term HA value for atrazine. It is, therefore, recommended that the adjusted DWEL for a 10-kg child of 0.05 mg/L (50 ug/L) and the DWEL for a 70-kg adult of 0.2 mg/L (200 ug/L) be used at this time as conservative estimates of the Longer-term HA values.

Lifetime Health Advisory

The Lifetime HA represents that portion of an individual's total exposure that is attributed to drinking water and is considered protective of noncarcinogenic adverse health effects over a lifetime exposure. The Lifetime HA is derived in a three-step process. Step 1 determines the Reference Dose (RfD), formerly called the Acceptable Daily Intake (ADI). The RfD is an esti-

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mate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious effects over a lifetime, and is derived from the NOAEL (or LOAEL), identified from a chronic (or subchronic) study, divided by an uncertainty factor(s). From the RfD, a Drinking Water Equivalent Level (DWEL) can be determined (Step 2). A DWEL is a medium-specific (i.e., drinking water) lifetime exposure level, assuming 100% exposure from that medium, at which adverse, noncarcinogenic health effects would not be expected to occur. The DWEL is derived from the multiplication of the RfD by the assumed body weight of an adult and divided by the assumed daily water consumption of an adult. The Lifetime HA is determined in Step 3 by factoring in other sources of exposure, the relative source contribution (RSC). The RSC from drinking water is based on actual exposure data or, if data are not available, a value of 20% is assumed. If the contaminant is classified as a Group A or B carcinogen, according to the Agency's classification scheme of carcinogenic potential (U.S. EPA, 1986b), then caution should be exercised in assessing the risks associated with lifetime exposure to this chemical.

Three studies were considered for the development of the Lifetime HA. A two-year dog feeding study (Woodard, 1964), a one-year dog feeding study Ciba-Geigy, 1987b) and a two-year rat oral feeding/oncogenicity study (Ciba-Geigy, 1986).

The first study in dogs (1964) reflected a NOAEL of 0.35 mg/kg/day and a LOAEL of 3.5 mg/kg/day that was associated with increased heart and liver weights in females. The new one-year dog study (1988) reflected a NOAEL of 0.48 mg/kg/day and a LOAEL of 4.97 mg/kg/day based on mild cardiac pathology intensified at the higher dose tested 33.65/33.8 (male/female) mg/kg/day. The two-year rat study (Ciba-Geigy, 1986) reflected a NOAEL at 3.5 mg/kg/day for systemic effect other than oncogenicity; however, this study indicated that atrazine caused mammary gland tumors at this dose level and above, no adverse effects were observed at the lowest dose tested, 0.5 mg/kg/day.

The 1964 dog study was initially used for the calculation of the RfD and the Lifetime HA. However, this study was partially flawed by the lack of information on the purity of the test material and by the inadequate documentation of the hematological data. Therefore, the recent one-year dog study (Ciba-Geigy, 1987b), using technical atrazine (97% ai.), is considered as a more adequate study for the calculation of the RfD and the Lifetime HA. The NOAEL in this study, 0.48 mg/kg/day, is also supported by the NOAEL of 0.5 mg/kg/day in the two-generation reproduction study (Ciba-Geigy, 1987a) and by the fact that no systemic effects or tumors were noted at this dose level in the two-year chronic feeding/oncogenicity study in rats (Ciba-Geigy, 1986). [Other studies: Woodard Research Corporation (1966) and Hazelton Laboratories (1961) identified long-term NOAEL values of 5 to 50 mg/kg/day and were not considered to be as protective as the dog studies for use in calculating the HA values for atrazine.]

Step 1: Determination of the Reference Dose (RfD)

$$RfD = \frac{0.48 \text{ mg/kg/day}}{(100)} = 0.005 \text{ mg/kg/day}$$

(rounded from 0.0048 mg/kg/day)

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where:

0.48 mg/kg/day = NOAEL, based on the absence of cardiac pathology or any other/adverse clinical, hematological, biochemical and histopathological effects in dogs.

100 = uncertainty factor, chosen in accordance with EPA or ODW/HAS guidelines for use with a NOAEL from an animal study.

Step 2: Determination of the Drinking Water Equivalent Level (DWEL)

$$DWEL = \frac{(0.0048 \text{ mg/kg/day})\ (70 \text{ kg})}{(2 \text{ L/day})} = 0.168 \text{ mg/L } (200 \text{ ug/L})$$

where:

0.0048 mg/kg/day = RfD (before rounding off to 0.005 mg/kg/day)

70 kg = assumed body weight of an adult.

2 L/day = assumed daily water consumption of an adult.

Step 3: Determination of the Lifetime Health Advisory

$$\text{Lifetime HA} = \frac{(0.168 \text{ mg/L})\ (20\%)}{10} = 0.003 \text{ mg/L } (3 \text{ ug/L})$$

where:

0.168 mg/L = DWEL (before rounding off to 0.2 mg/L)

20% = assumed relative source contribution from water.

10 = additional uncertainty factor, according to ODW policy, to account for possible carcinogenicity.

Evaluation of Carcinogenic Potential

- A study submitted by Ciba-Geigy Corporation (1986) in support of the pesticide registration of atrazine indicated that atrazine induced an increased incidence of mammary tumors in female Sprague-Dawley rats. These findings have been further confirmed in a briefing by Ciba-Geigy (1987) on this study.

- Atrazine was not oncogenic in mice (Ciba-Geigy, 1987c).

- Three closely related analogs: propazine, terbutryn and simazine are presently classified as Group C oncogens based on an increased incidence of tumors in the same target tissue (mammary gland) and animal species (rat) as was noted for atrazine.

- The International Agency for Research on Cancer has not evaluated the carcinogenic potential of atrazine.

- Applying the criteria described in EPA's guidelines for assessment of carcinogenic risk (U.S. EPA, 1986b), atrazine may be classified in Group C: possible human carcinogen. This category is used for substances with limited evidence of carcinogenicity in animals in the absence of human data.

VI. OTHER CRITERIA, GUIDANCE AND STANDARDS

- Toxicity data on atrazine were reviewed by the National Academy of Sciences (NAS, 1977), and the study by Innes et al. (1969) was used to identify a chronic NOAEL of 21.5 mg/kg/day. Although at that time it was concluded that atrazine has low chronic toxicity, an uncertainty factor of 1,000 was employed in calculation of the ADI from that study, since only limited data were available. The resulting value (0.021 mg/kg/day) corresponds to an ADI of 0.73 mg/L in a 70-kg adult consuming 2 L of water per day.

- Tolerances for atrazine alone and the combined residues of atrazine and its metabolites in or on various raw agricultural commodities have been established (U.S. EPA, 1986c). These tolerances range from 0.02 ppm (negligible) in animal products (meat and meat by-products) to 15 ppm in various animal fodders.

VII. ANALYTICAL METHODS

- Analysis of atrazine is by a gas chromatographic (GC) method, Method No. 507, applicable to the determination of certain nitrogen-phosphorus containing pesticides in water samples (U.S. EPA, 1988). In this method, approximately 1 L of sample is extracted with methylene chloride. The extract is concentrated and the compounds are separated using capillary column GC. Measurement is made using a nitrogen phosphorus detector. The method has been validated in a single laboratory. The estimated detection limit for the analytes in this method, including atrazine, is 0.13 ug/L.

VIII. TREATMENT TECHNOLOGIES

- Treatment technologies which will remove atrazine from water include activated carbon adsorption, ion exchange, reverse osmosis, ozone oxidation and ultraviolet irradiation. Conventional treatment methods have been found to be ineffective for the removal of atrazine from drinking water (ESE, 1984; Miltner and Fronk, 1985a). Limited data suggest that aeration would not be effective in atrazine removal (ESE, 1984; Miltner and Fronk, 1985a).

- Baker (1983) reported that a 16.5-inch GAC filter cap using F-300, which was placed upon the rapid sand filters at the Fremont, Ohio

water treatment plant, reduced atrazine levels by 30 to 64% in the water from the Sandusky River. At Jefferson Parish, Louisiana, Lykins et al. (1984) reported that an adsorber containing 30 inches of Westvaco WV-G8 12 x 40 GAC removed atrazine to levels below detectable limits for over 190 days.

- At the Bowling Green, Ohio water treatment plant, PAC in combination with conventional treatment achieved an average reduction of 41% of the atrazine in the water from the Maumee River (Baker, 1983). Miltner and Fronk (1985a) reported that in jar tests using spiked Ohio River water with the addition of 16.7 and 33.3 mg/L of PAC and 15-20 mg/L of alum, PAC removed 64 and 84%, respectively, of the atrazine. Higher percent removals reflected higher PAC dosages. Miltner and Fronk (1985b) monitored atrazine levels at water treatment plants, which utilized PAC, in Bowling Green and Tiffin, Ohio. Applied at dosages ranging from 3.6 to 33 mg/L, the PAC achieved 31 to 91% removal of atrazine, with higher percent removals again reflecting higher PAC dosages.

- Harris and Warren (1964) reported that Amberlite IR-120 cation exchange resin removed atrazine from aqueous solution to less than detectable levels. Turner and Adams (1968) studied the effect of varying pH on different cation and anion exchange resins. At a pH of 7.2, 45% removal of atrazine was achieved with Dowex 2 anion exchange resin and with $H_2PO_4^-$ as the exchangeable ion species.

- Chiam et al. (1975) reported that reverse osmosis, utilizing cellulose acetate membrane and a cross-linked polyethelenimine (NS-100) membrane, successfully processed 40% of the test solution, removing 84 and 98%, respectively, of the atrazine in the solution.

- Miltner and Fronk (1985a) studied the oxidation of atrazine with ozone in both spiked distilled and ground water. Varying doses of ozone achieved a 70% removal of atrazine in distilled water and 49 to 76% removal of atrazine in ground water.

- Kahn et al. (1978) studied the effect of fulvic acid upon the photochemical stability of atrazine to ultraviolet irradiation. A 50% removal of atrazine was achieved much faster at higher pH conditions than at lower pH conditions. In the presence of fulvic acids, the time needed for ultraviolet irradiation to achieve 50% removal was almost triple the time required to achieve similar removals without the presence of fulvic acids. Since fulvic acids will be present in surface waters, ultraviolet irradiation may not be a cost-effective treatment alternative.

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*Confidential Business Information submitted to the Office of Pesticide Programs.

J. RfD Document

006937

Guideline Series: 81-3

Reviewed by: Hank Spencer
Secondary reviewer: R.B. Jaeger
Date: 4/25/83

See MEMORANDA: "Atrazine Registration Standard" (Tox. Chapter dated 4/25/83).

Marion P. Copley D.V.M. /Date

DATA EVALUATION REPORT

CHEMICAL: Atrazine TOX. CHEM. NO.: 63

STUDY TYPE: Acute inhalation - rats

MRID NUMBER: 0002795 TOX. DOC.: 1983 Tox Reg Std

SYNONYMS: Atrozina, Tecnica

SPONSOR: Industria Prodotti Chimici

TESTING FACILITY: Hazleton Laboratories America, Inc.

TITLE OF REPORT: Acute inhalation - rats

AUTHOR(S): Frederick I. Reno

STUDY NUMBER(S): 915-100

REPORT ISSUED: April 1, 1975

CONCLUSION: $AILD_{50}$ for the nominal is > 167 mg/L
$AILD_{50}$ for actual exposure can not be determined.

Core Classification: Invalid for the reasons noted in the discussion.

DISCUSSION: This study and the original DER have been reevaluated and the study reclassified as core-invalid due to inadequate methodology, including, but not limited to the following:
Particle size was not determined, actual exposure to compound was not determined, atmospheric concentrations were not monitored.

006937

Acute Inhalation - Rats
Hazleton Laboratories America Inc.
Project #915 - 100 Dated: April 1, 1975
MRID: 00027095

Material Tested: Atrozina, Tecnica, (atrazine technical) (purity not stated).

Animal Tested: Male albino rat (254 g - 308 g).

Methods:

10 male rats were exposed to a single dose of 167 mg/L (nominal conc.) for 1 hr. in a 38L glass chamber. Air delivery was 10 L/min.

The rats were housed individually during exposure.

Observations:

Sacrificed on the 15th day after 14 days of daily observation.

Results:

Clinical signs - hypoactivity; excessive salivation; eye; nose, mouth discharge. At 2 days post exposure a slight brown crust exhibited around eyes, nose, mouth by day 4 signs had disappeared.

Tox. Cat. IV

LC_{50} > 167 mg/L 1 hr. (nominal)

Core:

Minimum. Purity of the technical grade is known to tbe 95% a.i. or greater.

81-6

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

006937

002816

Date: June 17, 1982

Subject: EPA Registration Number 201-411
Technical Atrazine

From: Deloris F. Graham CFG 6/24/80
PMB/TSS E 6/24/82

To: Robert Taylor
Product Manager (25)

Applicant: Shell Oil Company
Suite 200
1025 Connecticut Avenue, NW
Washington, DC 20036

Active Ingredient:
Atrazine (2-chloro-4-ethylamino
6-isopropylamine-S-triazine.....95%
Related Compounds.....5%

Background: Submitted a comparative Skin Sensitization Study to provide valid data to be substituted for the invalid skin sensitization data previously submitted. Study conducted by WIL Research Laboratories, Inc. Data not accessioned. Method of support not indicated.

Recommendation

(1) PMB/TSS finds these data acceptable to support conditional registration of this product.

Label:

(1) No additional label comments.

Review:

(1) Comparative Skin Sensitization using Technical Atrazine (Code 7-15-0-0) VB-304-398 and (Code 7-14-0-0) 2401-143); Project #WIL-1214-78; March 21, 1979.

Procedure: Test groups consisted of 5 M and 5 F guinea pigs and 4 control groups consisting of 3 M and 2 F guinea pigs in two groups and 3 F and 2 M in the other two groups. Each of the 11 groups received one of the following

1/05

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treatments: Vehicle Control - 100% ethanol; Positive Control - 0.1%, 2,4 dinitrochlorobenzene in ethanol; Technical Atrazine (Code 7-15-0-0) 10% w/v solution in ethanol; Technical Atrazine (Code 7-14-0-0) 10% w/v solution in ethanol; Vehicle Control - distilled water; Technical Atrazine (Code 7-15-0-0) nonirritating slurry with water; Technical Atrazine (Code 7-14-0-0) nonirritating slurry with water; Technical Atrazine (Code 7-15-0-0) 10% w/v solution in ethanol; Technical Atrazine (Code 7-14-0-0) 10% w/v in ethanol; Technical Atrazine (Code 7-15-0-0) nonirritating slurry with water; Technical Atrazine (Code 7-14-0-0) nonirritating slurry with water. There were three sensitizing doses given topically on shaven areas, once per day for three consecutive days. Following a four day rest period after the last sensitizing dose, the challenge dose was administered. A 0.5 ml or 0.5 g dose of the appropriate material was used. Observation made 24 hours after the first sensitizing dose and after the challenge dose.

Results:

Vehicle Control Group 1 - 100% Ethanol

Four guinea pigs exhibited slight erythema, five exhibited minimal erythema, and one exhibited no response to the application of the sensitizing dose. Five guinea pigs exhibited slight erythema. Two exhibited minimal erythema and three exhibited no response to the application of the challenge dose.

Positive Control Group 2 - 2,4 dinitrochlorobenzene-0.1% (w/v) solution in ethanol

Three guinea pigs exhibited slight erythema, three exhibited minimal erythema and four exhibited no response to the application of the sensitizing dose. Four guinea pigs exhibited moderate erythema and edema, three exhibited moderate erythema, two exhibited slight erythema and one exhibited mirimal erythema to the application of the challenge dose.

Test Group 3 - Technical Atrazine (Code 7-15-0-0)10% (w/v) solution in ethanol

Four guinea pigs exhibited slight erythema, four exhibited no response to the application of the sensitizing dose. One guinea pig exhibited moderate erythema with slight edema, two exhibited minimal erythema and seven exhibited no response to the application of the challenge dose.

Test Group 4 - Technical Atrazine (Code 7-14-0-0) 10% (w/v) solution in ethanol

Four guinea pigs exhibited slight erythema, five exhibited minimal erythema and one exhibited no response to the application of the sensitizing dose. Five guinea pigs exhibited slight erythema, two exhibited minimal erythema and three exhibited no response to the application of the challenge dose.

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Vehicle Control Group 5 - Distilled Water

None of the guinea pigs exhibited any response to the application of the sensitizing dose. One guinea pig exhibited minimal erythema and nine exhibited no response to the application of the challenge dose.

Test Group 6 - Technical Atrazine (Code 7-15-0-0) nonirritating slurry

None of the guinea pigs exhibited any response at the application of the sensitizing dose or the challenge dose.

Test Group 7 - Technical Atrazine (Code 7-14-0-0) nonirritating slurry

None of the guinea pigs exhibited any response to the application of the sensitizing dose. Three guinea pigs exhibited minimal erythema and seven exhibited no response to the application of the challenge dose.

Groups 8 through 11 were virgin irritation control groups to be applied and scored only at the primary challenge. None of the previously mentioned groups exhibited any response to the application.

Based on the results submitted, Technical Atrazine (Code 7-15-0-0) and (Code 7-14-0-0) are nonsensitizing.

Study Classification: Core minimum. Observations at 24 and 48 hours after each application.

Toxcitity Category: Nonsensitizing

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006937 82-1 006937

Reviewed by: Marion P. Copley, D.V.M., D.A.B.T. 11/9/88
Sect.2, Acting Section Head, TB I (IRS) (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. 11/21/88
Acting Chief, TB I (IRS) (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 90-day feeding - dog TOX. CHEM NO: 63

MRID NO.: 00163339

TEST MATERIAL: Atrazine

SYNONYMS: Atranex

STUDY NUMBER: T-635

SPONSOR: Agan Chemical Manufacturers Ltd.

TESTING FACILITY: WARF Institute, Inc., Madison, Wisconsin

TITLE OF REPORT: 90-Day subacute feeding study of Atranex in dogs

AUTHOR(S): M. Tisdel, D. Harris

REPORT ISSUED: Not specified, Initiated Jan. 12, 1977

CONCLUSION:

NOEL < 200 ppm (5 mg/kg/day) (low dose tested)
LEL ≤ 200 ppm (5 mg/kg/day) based on body weight gain depression in the **males**.

In addition at 623 ppm (15.8 mg/kg/day) and above in **males**, there was a slight decrease in RBC, HCT and HGB. There was also a mild to total arrest of spermatogenesis.
At 2000 ppm (50 mg/kg/day) in **males**, there was decreased food consumption; in **females**, there was body weight loss, decreased food consumption, and a slight decrease in RBC, HCT and HGB.

Classification: CORE-SUPPLEMENTARY

Special Review Criteria (40 CFR 154.7) could not be determined since a NOEL was not established.

A. MATERIALS:

1. Test compound: Atrazine technical, Description - not given, Batch # - 7003, Purity - not given %.

2. Test animals: Species: dog, Strain: beagle, Age: not given, Weight: not given, Source: Ridglan Farm, Inc, Mount Horeb, Wisconsin, animals were given a 2 month acclimatization period.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned to test groups based on weight, sex and litter identification (see table 1).

TABLE 1 Experimental Design

Test Group	Dose in diet (PPM)	Est. dose based on 0.025 mg/kg per PPM (mg/kg/day)	Main Study 3 months male	female
1 Cont	0	0	4	4
2 Low (LDT)	200	5	4	4
3 Mid (MDT)	632	15.8	4	4
4 High (HDT)	2000	50	4	4

2. Diet preparation

Diet preparation, storage and analysis for homogeneity, stability and concentration were not discussed.

3. Animals received food (Purina Canine Diet) ad libitum for 1 hour daily and water ad libitum.

4. Statistics - The procedures utilized in analyzing the numerical data are not listed. Means and standard deviations appear to have been used for some numerical parameters, ie. body weight and food consumption. Organ weights were compared using the T-Test.

5. A signed quality assurance statement was not present. In addition, there was no signature page.

C. METHODS AND RESULTS:

1. Observations:

Animals were inspected daily for signs of toxicity and mortality.

Toxicity/Mortality (survival) - There were no mortalities or treatment related clinical signs observed during the study.

2. Body weight

Animals were weighed at study initiation, then weekly and again at termination.

Results - MALES - As can be seen in table 1, there was a decrease in body weight gain and % body weight gain in all groups of males treated with atrazine. This decrease was dose related. Two dogs in each of the LDT, MDT and HDT actually lost weight during the study. Statistics however, were not done for these values. FEMALES - Only the HDT females had a decreased weight gain (actually a weight loss of 6 %, compared to a gain of 24-28% in the other 3 groups).

TABLE 2 Body weight (gm) and % weight gains for 13 weeks

Conc in diet PPM	male day 0	male day 90	male gain	male % gain	female day 0	female day 90	female gain	female % gain
Cont.	8063	10375	2312	29%	6588	8150	1562	24%
200	7600	8539	939	12%	6375	8075	1700	27%
632	6788	7225	437	6%	5538	7075	1537	28%
2000	7738	7363	-375	-5%	6975	6575	-400	-6%

3. Food consumption and compound intake

Consumption was determined daily and reported as weekly totals of mean daily diet consumption. Food consumption in terms of body weight, food efficiency and compound intake were not calculated.

Food consumption - Food consumption was only decreased (estimated at 30 % less than controls) in the HDT males and females. Values in the other groups were variable and could not be attributed to treatment due to the small sample size (n=4). Statistics were not done on these values.

4. Ophthalmological examinations were not reported.

5. Blood was collected before treatment and at 0, 4, 8, and 13 weeks for hematology and clinical analysis from all animals (fasting not specified). The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
	Platelet count*		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies

Results - As can be seen in table 3, there appeared to be a slight decrease in RBC, Hct and Hgb in males at the MDT and HDT and in females at the HDT. Other changes were within expected ranges for the age and species or there was too much within group variation to make any conclusions.

TABLE 3 Select hematology (13 week)

Conc in diet PPM	HCT (%)	HGB (G%)	RBC 10^6 cells/mm^3	HCT (%)	HGB (G%)	RBC 10^6 cells/mm^3
		MALES			FEMALES	
Cont.	43	15.3	6.92	41	15.2	6.97
200	41	14.8	6.57	42	15.0	6.98
632	35	13.0	5.57	39	14.0	6.19
2000	33	12.2	5.46	34	12.8	5.80

b. Clinical Chemistry

X		X	
	Electrolytes:		Other:
X	Calcium*	X	Albumin*
X	Chloride*		Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorous*		Cholesterol*
X	Potassium*	X	Alb/Globulin ratio
X	Sodium*	X	Glucose*
	Enzymes	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum Protein (TP)*
	Cholinesterase (ChE)#		Triglycerides
	Creatinine phosphokinase*^		Serum protein electrophores
	Lactic acid dehydrogenase (LAD)		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic and chronic studies
# Should be required for OP
^ Not required for subchronic studies

Results - There were no treatment-related changes in clinical chemistry values.

6. Urinalysis^

Urine was collected (fasting not specified) animals at 0, 4, 8 and 13 weeks. The CHECKED (X) parameters were examined.

X		X	
	Appearance*	X	Glucose*
	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
	Sediment (microscopic)*		Nitrate
X	Protein*		Urobilinogen

^Not required for subchronic studies
* Required for chronic studies

Results - There were no treatment-related changes.

7. Sacrifice and Pathology

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. (x) Tissues were collected but not examined. The (XX) organs, in addition, were weighed.

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
	Tongue		Aorta*	X	Brain*+
X	Salivary glands*	XX	Heart*	x	Periph. nerve*#
	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen	x	Eyes (optic n.)*#
X	Jejunum*		Thymus*	Glandular	
X	Ileum*	Urogenital		XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+		Lacrimal gland#
	Colon*	X	Urinary bladder*		Mammary gland*#
	Rectum*	XX	Testes*+		Parathyroids*++
XX	Liver *+		Epididymides	XX	Thyroids*++
X	Gall bladder*	X	Prostate	Other	
X	Pancreas*		Seminal vesicle	X	Bone*#
Respiratory		XX	Ovaries*+		Skeletal muscle*#
X	Trachea*	X	Uterus*	x	Skin*#
X	Lung*			X	All gross lesions and masses*
	Nose				
	Pharynx				
	Larynx				

(key on next page)

* Required for subchronic and chronic studies.
# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement.
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

a. Organ weight - There appeared to be a slight increase in kidney weight (relative) at the HDT in both males and females. The biological significance of this can not be determined since: 1) these values were not significant at $p < 0.05$ level for the T-Test, and 2) there were no unusual renal histologic findings. Absolute testes weight were decreased about 30 % in the HDT males while relative weights were only decreased about 8%. All other organ weights were similar to controls.

b. Gross pathology - There were no treatment-related changes noted at necropsy.

c. Microscopic pathology

All MDT and HDT males had mild to total arrest of spermatogenesis. In addition, some testes had abnormal cells or degenerative tubules. There were no other histologic changes related to treatment.

D. DISCUSSION:

Four beagles/sex/group were exposed for 90 days to atrazine technical in the diet at either 0, 200, 632 or 2000 ppm, resulting in approximate doses of 0, 5, 15.8 and 50 mg/kg/day, respectively.

There were no overt signs of toxicity or mortality due to atrazine treatment. Although the company states that body weight was only affected in the high dose males and females, the data indicate that body weight was decreased at all 3 atrazine treatment levels in males and high dose females. Even when body weight was evaluated for each individual animal (rather than as means), there appeared to by no NOEL for males. As mentioned in the results, 2 males in each atrazine treatment group had weight losses while the lowest weight gain in controls for the 90 day period was 12 %. Changes at the weekly intervals were less informative than the monthly or 90 day changes due to the small sample size (n=4). Body weight losses at the high dose were consistent with decreased food consumption at this dose. The other food consumption data was difficult to interpret since it was reported as g/dog rather than adjusted for body weight (g/kg body weight).

Slight decreases in hematologic indices (RBC, HCT and HGB) suggested a possible anemia in the MDT and HDT males and HDT females. Although this is consistent with findings in other studies, the biological significance could not be determined conclusively since the changes and sample size were small. All other measured laboratory parameters were similar to control values.

Histologic changes were limited to a mild to total decrease in spermatogenesis in all mid and high dose males. The HDT decrease in testicular weight, although consistent with these histologic changes, may have been due in part, or completely to decreased body weight. Other organs (weight and histopathology) appeared unaffected by treatment.

Since this study report was completed in the 1970s, it does not contain information now required in every report. The following items were not present or incompletely reported:

1) date report completed
2) signature page
3) signed quality assurance statement
4) purity of the test compound
5) test diet preparation (frequency) and storage information
6) analysis of homogeneity, stability and concentration of test diet
7) summary of statistical methods used
8) test compound intake, based upon body weight and feed consumption

Due primarily to the lack of a NOEL in the males for body weight depression and the other deficiencies noted above, this study is assigned a core-classification of SUPPLEMENTARY. In addition, at 623 ppm (15.8 mg/kg/day) and above in males, there was a slight decrease in RBC, HCT and HGB. There was also a mild to total arrest of spermatogenesis. At 2000 ppm (50 mg/kg/day) in males, there was decreased food consumption; in females, there was body weight loss, decreased food consumption, and a slight decrease in RBC, HCT and HGB.

NOTE: Although electrocardiography is not required in subchronic dog studies, this is one of the more sensitive parameters reported in an acceptable chronic dog study for atrazine. Therefore, this study should not be used to determine potential subchronic cardiac toxicity from atrazine even though there were no histological cardiac changes.

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4-11-88

Reviewed By: Irving Mauer, Ph.D. TB Project No.: 8-0320
Section VI, Toxicology Branch (TS-769C) Date:
Secondary Reviewer: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch (TS-769C)
Judith W. Hauswirth 4/20/88

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Atrazine Caswell No.: 063
TOX Chem No.: 080803

Study Type: Chronic (52-Week) Feeding - Dog

Citation: Atrazine Technical - 52-Week Oral Feeding in Dogs (MIN 852008)

Accession No.: 404313-01 (3 volumes) ← MRID

Sponsor: Ciba-Geigy, Corporation, Greensboro, NC

Testing Lab.: Ciba-Geigy, Division of Toxicology/Pathology
Summit, NJ

Study No.: 852008 (Tox./Path. Rpt. No. 87048)

Study Date: October 27, 1987

TB Conclusions/Evaluation:

CORE MINIMUM DATA. Although the mid-dose (150 ppm) was proposed by the testing laboratory as the NOEL, a number of minimal cardiac changes were found in a few animals at this intermediate dose. Hence, TB regards the LDT (15 ppm) as the NOEL, until more exactly defined between 15 and 150 ppm.

Dietary doses administered: 0, 15, 150, and 1000 ppm.

Intake equivalent: mg/kg/day

Male - 0, 0.48, 4.97, and 33.65

Female - 0, 0.48, 4.97, and 33.80

DETAILED REVIEW

Test Article:

Atrazine technical (Batch FL 850612); nature and purity not stated here (but reported elsewhere as 97 percent); mixed in canine feed (Purina No. 5007) for oral feeding. Samples of the admixture were monitored at regular intervals for contaminants, stability, homogeneity, and atrazine concentration.

Procedures:

Purebred 5-month-old male and female beagle dogs (Marshall Farms, North Rose, NY) were acclimated to the laboratory for 1 month, then assigned randomly to four groups, which received feed containing 0 (feed only, six animals/sex), 15 ppm (four animals/sex), 150 ppm (four/sex), and 1000 ppm (six animals/sex) test article.

The animals were observed daily, and body weight and food consumption determined pretreatment, and weekly during the first 13 weeks of treatment, then monthly thereafter. Auditory and ophthalmoscopic (by Fison Indirect Ophthalmoscope) examinations were performed during the pretreatment period and quarterly during treatment, as were electrocardiographic (EKG) tracings (10 leads). Seven hematological parameters (hemoglobin, hematocrit, rbc, wbc, differential, platelets, and prothrombin time) were also measured during the pretreatment period and quarterly thereafter, in addition to reticulocytes and Heinz bodies. Fifteen serum chemistry values (BUN, creat, SGOT, SGPT, alk phos, glu, tot bili, tot chol, inorg phos, Na, K, Ca, Cl^-, tot prot, alb, glob, and A/G ratio) including CPK and LDH, and nine urinalysis values (spec grav, pH, prot, glu, bili, urobili, ket, occ blood and micro) were also determined according to the same sampling schedule. All surviving animals were necropsied during week 53; complete necropsies were also carried out on any animals that died during the study period.

A complete roster of tissues, according to Test Guideline directives, was fixed in 10% neutral buffered formalin for microscopic examination:

Adrenal (2)	Kidney (2)	Salivary Gland
All Gross Lesions incl. Tissue Masses	[Larynx/Pharynx]	Skin
	Liver	Spinal Cord:
Aorta	Lung	cervical
Brain:	Lymph node:	lumbar
cerebrum	Axillary	thoracic
cerebellum	Mesenteric	Spleen
medulla/pons	Mammary gland (F)	Sternum w/marrow

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Cecum	Muscle, thigh	Stomach
Colon	Nerve, sciatic	Testis w/epididymis (2)
Duodenum	Ovary (2)	Thymus, when present
Esophagus	Pancreas	Thyroid w/parathyroid (2)
Eyes w/optic nerve	Pituitary	Tongue
[Femur w/joint]	Prostate	Trachea
Gallbladder	Rectum	Urinary Bladder
Heart	[Rib at costochondral joint]	Uterus
Ileum		Vagina
Jejunum		[Vertebra]

Absolute organ weights (brain, heart, kidney, liver, ovary, pituitary, spleen, testis with epididymis, and thyroid/parathyroid) were determined, and relative weights calculated using a computer system. Gross pathology data were initially recorded manually, but later entered into the NO3 pathology data base. A detailed examination of the hearts of all animals sacrificed at study termination was performed, including measurement of left and right ventricular wall thickness, in order to augment the description of the nature and severity of any cardiac lesions. [NB: Cardiac toxicity was the subject of a flagging statement in the final report of this study (Volume 1, page 4 of 1405).]

Data on body and organ weights, feed consumption, clinical laboratory values, and EKG tracings were stored in the Beckman toxicology system data base (TOXSYS) in the IBM 4361 mainframe computer, and analyzed separately for each sex by a two-tailed Dunnett's multiple comparisons test ($p < 0.05$ and < 0.01) at each time point during treatment, in order to detect any differences between each treatment mean and control. During the pretreatment period, an F-test for testing equality of all treatment group means was performed. Nonparametric versions of these procedures (based on rank rather than numerical values) were employed on parameters not normally distributed (e.g., highly skewed). Supplemental statistical analyses were necessary where the significant deviations were detected via diagnostic procedures. These included data transformation, nonparametric tests, and tests without assuming homogeneity of group variances.

Results:

Chemical Analysis/Dosimetry

Routine (monthly) chemical analysis of feed admixtures indicated that the concentrations of the test material were within ± 10 percent of target concentrations (92 to 107 percent, Appendix 9.8). The admixtures were stated to be stable for at least 21 days at room temperature (92 percent of target concentrations for the "15 ppm" test formulation, Appendix 9.7). (NB: The same tabulation indicates, however, a 99 percent target stability for the high-dose admixture, 1000 ppm.) Analysis of

multiple subsamples during week 1 of treatment revealed atrazine was homogeneously distributed throughout the feed admixtures (relative standard deviations were 2, 1.3, and 1.7 percent, respectively, for the three dosage groups: 15, 150, and 1000 ppm, Appendix 9.9).

Based on feed consumption and average-group mid-period body weight (Table 8.4, Appendix 9.1.4, discussed below), the daily doses and ranges (from Table 8.3) were calculated as follows:

Sex	Group	Dietary Concentration (ppm)	Mean Daily Dose (mg/kg/day)	Range (mg/kg/day)
M	2	15	0.48	0.4 - 0.6
	3	150	4.97	4.3 - 5.7
	4	1000	33.65	20.1 - 38.0
F	2	15	0.48	0.4 - 0.6
	3	150	4.97	4.2 - 6.0
	4	1000	33.80	23.1 - 39.3

Mortality/Clinical Signs

Three animals had to be sacrificed during the study in a moribund condition: one 150 ppm male (14 M) on day 75; one 1000 ppm female (39 F) on day 113; and one 1000 ppm male (16 M) on day 250 (Table 8.1, Appendix 9.1.1). Control and 15 ppm animals survived the entire study period without incident. The authors considered the moribund condition of 14 M to have developed spontaneously, unrelated to atrazine treatment. This animal lost 3 kg and became cachectic early in the second month of treatment, following prior bouts of hypoactivity, bloody discharge from the penis, fecal changes (bloody, mucoid, and/or soft), mydriasis, and reduced pupillary response. Clinical laboratory changes included elevated white cell count, increased serum globulin level, and depressed erythroid parameters. Principal histopathological findings were stated to be consistent with published reports of disseminated fibrinoid necrotic arteritis (termed "polyarteritis nodosa," or, alternatively, necrotizing vasculitis), considered a spontaneous disease in the dog, involving meningeal, coronary, renal, and other blood vessels (mesenteric, testicular, etc.).

Whereas the authors considered the moribund condition of 39 F (1000 ppm) to be related to compound administration, they considered that of 16 M receiving the same compound level unrelated to treatment. 39 F displayed a syndrome of clinical changes including an abnormal EKG profile, ascites and cachexia, referable to cardiopathy. Pathological changes included abundant clear ascites fluid; cardiac dilatation, and liver adhesions. Histologic findings included degenerative lesions (both atrophy

and myelosis) of the atrial myocardium, as well as hepatic centrilobular necrosis. On the other hand, 16 M manifested a persistent and progressive dermatitis with development of sores and scabs, and failure to gain weight associated with low food consumption, but none of the clinical signs referable to cardiac insufficiency. Necropsy findings included skin lesions associated with Demodex sp. mites, but also degenerative lesions in both atrial myocardia. Hence, while the moribundity of 16 M was considered to be due to the debilitating, progressive dermatitis caused by mites, the authors admit the possibility that atrazine could have contributed to the debilitated state, because of the presence of "compound-related myocardial lesions."

Among animals on 1000 ppm atrazine, treatment-related clinical signs attributable to atrazine-induced myocardial degeneration (discussed below) included ascites (one each high-dose male, 15 M, and female 39 F), cachexia (one male, 14 M, 150 ppm; 1 female, 39 F, 1000 ppm) and labored/shallow breathing (15 M, 1000 ppm). Both of these high-dose animals as well as three others fed at 1000 ppm (19 M, 20 M, and 40 F) had EKG and/or morphologic evidence of heart disease (characterized as irregular heartbeat, tachycardia, and increased heart rate), in contrast to the mid-dose dog, 14 M, which did not. These treatment-related clinical signs correlated to pathologic findings attributed to atrazine administration.

A variety of other clinical signs, considered by the authors to be spontaneous findings commonly found in laboratory dogs were recorded (see Report Table 8.1 attached to this review). Since they occurred randomly and were not dose-related, they were considered to be unrelated to atrazine treatment.

Body Weight/Food Consumption

High-dose males and females ate less feed from the first week of treatment, which caused (at least in part) a parallel reduction in body weight or body weight gain (Figures 7.1, 7.2, 7.3, and 7.4; Table 8.4; Appendix 9.1.4). Compared to baseline (pretreatment) values, percent body weight gain at study termination for the 1000 ppm group was +29.7 percent for males and 21.5 percent for females, whereas for controls the gain was 46.6 and 35.6 percent, respectively (Report Table 8.2, attached to this review). However, only the change in males was significant, at $p < 0.05$, using the two-tailed Dunnett's test. Treatment-related, statistically significant reductions in food consumption were found in high-dose males and females during the first quarter of the study period (Table 8.4). Where differences were not significant, food consumption was recorded as consistently less than in controls. No statistically significant reduction in body weight or percent body weight gain and food consumption were recorded in males or females ingesting feed containing 15 or 150 ppm atrazine.

Ophthalmoscopy

No compound-related ocular changes were found on ophthalmoscopic examinations (Appendix 9.2). A summary of individual ophthalmoscopic findings revealed comparable incidences and types of changes in control as in test groups.

Electrocardiography

Treatment-related EKG changes were stated in the text of the report to be found only in the high-dose groups at various time points of recording (Appendix 9.3). Statistically significant findings in males were recorded for increased heart rate on days 85, 175, and 361; decreased P-II (height of P wave) on days 85, 175, 267, and 361; decreased PR-interval time on day 361; and decreased QT-interval time on days 267 and 361. Statistically significant findings in females consisted of decreased P-II on days 85, 175, 267, and 361; decreased PR on day 175; and increased heart rate on day 175.

The authors noted other statistically significant EKG changes in all test groups, but these were considered "incidental and unrelated to treatment." They included decreased mean electrical axis at day 175 and increased P-II and PR at day 267 in low-dose females; increased R waves at day 361 in mid-dose, and at days 267 and 361 in high-dose males; but also significantly decreased P-II of 0.200 mV ($p < 0.05$) in mid-dose (150 ppm) females at day 175. (This is the same change considered treatment-related in the high-dose group, with a mean value of = 0.100 mv at day 175 in high-dose females, $p < 0.01$.) The value in 150 ppm females was stated to be within the normal background range of values for the species. (Background values were not provided in the report, however, but the concurrent control value was given as 0.267 mV.) The staff cardiologist for Ciba-Geigy also noted arrythmia (atrial fibrillation) in four high-dose dogs (two males and two females) at various time points, and atrial premature complexes in one high-dose female at day 361 (Appendix 9.3).

Hematology

Treatment-related changes in hematological values were reported to have occurred in the high-dose group only (Table 8.5 and Appendix 9.1.5). These changes consisted of slight but statistically significant reductions in erythroid parameters (red cell count, hemoglobin, and hematocrit) in males only throughout the study (considered secondary to body weight depression), and mild increases in platelet counts in both sexes (said to be "minimal" by the authors), and of unclear toxicologic significance, since this increase did not correlate to any pathologic observation. Additionally, sporadic statistically significant

alterations in other hematologic parameters, considered by the authors to be toxicologically unimportant, occurred in males of all test groups (MCV, WBC, eosin) and in mid- and high-dose females (lymp mono, WBC, MCHB, and MCHC). All these changes were considered "spontaneous and unrelated to treatment," since neither dose-response nor consistent time-response occurred, they were not associated with any morphologic findings, and group mean values were generally within the range of concurrent control values, as well as historical background for differential white blood cell counts (stated as "MIN" and "MAX" in Table 8.5).

Biochemistry

The only compound-related biochemical changes reported were slight decreases in total protein and albumin, statistically significant ($p < 0.05$) for high-dose males and considered secondary to reduced feed consumption (Table 8.6 and Appendix 9.1.6). Other statistically significant differences occurred sporadically in low- and high-dose males and females, and were considered spontaneous changes unrelated to treatment, since they were neither dose- nor time-related, but marginal and within concurrent control values. Among low-dose animals, findings consisted of increased total bilirubin in males and increased phosphorus in females; in high-dose groups calcium was decreased and chloride increase in males, while females had increased sodium and glucose.

Urinalysis

No stated compound-related changes in urinary values were found, although several sporadic statistically significant differences occurred in all test groups (Table 8.7 and Appendix 9.1.7). Low-, mid-, and high-dose females had nondose-related increased numbers of epithelial cells at day 175 only; in addition, high-dose females had decreased protein at day 270. Decreased crystals and specific gravity were noted in mid-dose males at day 89; on the other hand, high-dose males manifested increased crystals at day 89, decreased epithelial cell number at day 357, and decreases in WBC at day 175. All these changes were considered spontaneous, with no consistency, and within the range of concurrent control values observed in this study.

Organ Weights

Treatment-related, statistically significant changes in organ weights were recorded for high-dose animals, specifically decreases in absolute (but not relative) heart weight in females, and increased relative liver weight (both body and brain weight ratios) in males (Table 8.8 and Appendix 9.1.8). The cardiac changes were considered a direct effect of atrazine administration, while the liver weight change was primarily due to one animal (15 M) and considered secondary to ascites. Other

statistically significant changes in organ weights occurred in mid- and high-dose animals, but these were considered unrelated to treatment. For example, mid-dose females manifested marginal increases in absolute heart weight and heart/brain ratios inconsistent with the treatment-related decreases at the higher dose. High-dose females had increased relative (to body weight) ovary weights, consistent with stages of estrus rather than atrazine administration.

Gross Pathology

Gross pathological changes considered to be related to atrazine administration were found in the majority of high-dose survivors (4/5 males and 5/5 females) and consisted mainly of moderate to severe dilatation of right and/or left atria; less common cardiac changes included fluid-filled pericardium and enlarged heart in three males (Report Tables 3 and 4 of Appendix 9.4, attached to this review). Secondary changes included abdominal ascites and liver adhesions, the most severe noted in two animals (15 M and 39 F), one of which was sacrificed moribund before study termination (39 F).

Microscopic Pathology

Microscopic findings were correlated to the gross changes, cardiac lesions occurring most often and restricted to high-dose males and females (Table 5 of Appendix 9.4, attached). The principal histologic lesion observed was atrophy and myolysis of the atrial myocardium, with atrial edema. Less common lesions in high-dose animals included centrilobular hepatic necrosis (two females) and serous lymphadenitis of the mesenteric lymph node (one male and one female). These treatment-related gross and histologic changes were also correlated to EKG abnormalities.

Summary

In summary, the authors concluded that treatment-related effects of atrazine feeding were found only at the highest dietary level (1000 ppm = 33.65 mg/kg/day for males; 33.80 mg/kg/day for females), and included (as taken directly from the Final Report):

1. At least one death (female) [and possibly two, if one includes 16 M];
2. Cachexia and ascites;
3. Reduction in body weight and percent body weight gain;
4. Reduction in food consumption;
5. Irregular heartbeat and increased heart rate;

6. EKG alterations such as increased heart rate, decreased P-IF values, atrial premature complexes, and atrial fibrillation;

7. Slight changes in hematological parameters such as decreased erythroid values and increased platelet counts;

8. Slightly decreased serum (total) protein and albumin;

9. Slightly decreased absolute heart weight in females and slightly increased relative liver weights in males;

10. Moderate to severe cardiac lesions consisting primarily of dilatation of right and/or left atria and myocardial degeneration (atrophy, myelosis) of the atria. The cardiac lesions were considered direct effects of atrazine administration, whereas many of the additional findings (clinical, hepatic, etc.), were considered secondary.

From these results the authors proposed that the NOEL in this dog study was 150 ppm (actual intake, 4.97 mg/kg/day for both sexes), and based upon the cardiotoxicity observed, the MTD was exceeded at 1000 ppm.

A Quality Assurance (QA) Statement was present, attesting to repeat inspections/audits of this study, and signed by the Director of QA/Regulatory Compliance, October 27, 1987.

TB Evaluation: Core-Minimum Data

Doses administered: 0, 15, 150, and 1000 ppm (equivalent to measured intakes of: 0, 0.48, 4.97, and 33.65/33.80 [M/F] mg/kg/day.

The most significant effect of atrazine administration described in this 1-year dog study was the syndrome of cardiopathy, featuring discrete myocardial degeneration and most prominently found in the test group receiving the highest concentration of dietary atrazine, 1000 ppm (equivalent to actual intakes of 33.65 mg/kg/day for males, 33.80 mg/kg/day for females). Clinical signs referable to cardiac toxicity, such as ascites, cachexia, labored/shallow breathing, and abnormal EKG, were first observed as early as 17 weeks into the study. Gross pathological examination revealed moderate to severe dilatation of the right atrium (and occasionally the left atrium), microscopically manifest as atrophy and myelosis (degeneration of the atrial myocardium). The authors proposed that the NOEL was 150 ppm (4.97 mg/kg/day) for both sexes.

While conceding that the MTD was exceeded at 1000 ppm, we

question whether 150 ppm represents a valid no-effect level. Two mid-dose males manifested some cardiac involvement, which was discounted by the authors as not treatment-related. Animal 12 M had a "moderate" degree of dilatation of the right atrium, combined with "minimal" dilatation of the left atrium, plus a "pale lesion of the epicardium of the left ventricle" on gross examination (but it was asserted that no microscopic atrial lesions were found). Animal 14 M was sacrificed moribund during the 11th treatment week manifesting clinical signs such as hypoactivity and cachexia (among other changes). Gross pathological examination of this animal revealed "red" right atrium, histologically manifest as a "thickened" atrium with edema. The cause of death was stated to be consistent with disseminated arteritis, reported to occur spontaneously in beagle dogs, and termed "polyarteritis nodosa." Additional support for considering 150 ppm an effect level is provided by significantly decreased P-II waves in mid-dose females at day 175 of the study.

If these cardiac changes at the mid-dose level represent the lower tier of atrazine effects, then this intermediate dose should be considered an effect level (LEL); thus, the next lowest dose level (15 ppm (0.48 mg/kg/day), becomes the NOEL. This would conform to the TSCA Test Guidelines, which advise that the intermediate dose produce a low level of toxicity, and there be a gradation of effects from the appropriate spacing of doses.

Attachments

ATRAZINE 080803

Page_____ is not included in this copy.

Pages 107 through 120 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

.A. REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfDo) 006937

nical -- Atrazine
RN -- 1912-24-9
WELL -- 063
Line: 08/02/88

I.A.1. ORAL RfD SUMMARY

itical Effect	Experimental Doses*	UF	MF	RfD
gnificant decreased II waves in females day 175 and rdiac toxicity seen two dogs Year Dog Feeding udy ba-Geigy, 1987	NOEL: 15 ppm (0.48 mg/kg/day) LEL: 150 ppm (4.97 mg/kg/day)	100	1	5E-3 mg/kg/day
creased body ights of pups of neration on the 2nd stnatal day 21 Generation Rat roduction Study ba-Geigy, 1987	NOEL: 10 ppm (0.5 mg/kg/day) LEL: 50 ppm (2.5 mg/kg/day)			

nversion Factors: 1 ppm = 0.025 mg/kg/day (assumed dog food consumption)
nversion Factors: 1 ppm = 0.05 mg/kg/day (assumed rat food consumption)

I.A.2. PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

azine Technical - 52-Week Oral Feeding Study in Dogs
a-Geigy, Division of Toxicology/Pathology Summit, NJ
a-Geigy Corporation
dy No. 852008 (Tox./Path. Rpt. No. 87048); October 27, 1987
Accession No. 404313-01

Purebred 5-month old male and female beagle dogs were acclimated to the oratory for 1 month, then assigned randomly to four groups, which received l containing 0 (feed only, six animals/sex), 15 ppm (4 animals/sex), 150 (4 animals/sex), and 1000 ppm (6 animals/sex) [0, 0.48, 4.97, 33.65 (M), (F) mg/kg/day] for 52 weeks. The LEL for this study is 150 ppm based a statistically signifcant decreased P-II waves in females at day 175 of dy and cardiac toxicity in two male dogs were observed. At the highest e tested (1000 ppm), EKG alterations such as increased heart rate, eased P-II values, atrial premature complexes, and atrial fibrillation moderate to severe cardiac lesions (dilatation of the right atrium, phy and myelosis) were observed.

iero, J.; Youreneff, M.; Giknis, M.; Yau, E.T. 006937
neration Reproduction Rat Study
arch Department, Pharmaceuticals Division, Ciba-Geigy Corporation
-Geigy Corporation, Agricultural Division
y No. 852063; November 17, 1987
No. 40431303

One hundred twenty male and 120 female rats were randomly distributed into eatment groups [0, 10, 50, 500 ppm (0, 0.5, 2.5, and 25 mg/kg/day)]. rats were placed on the control and test diets at 47 days of age and les at 48 days of age. They were maintained on these diets for a period 0 weeks prior to mating. Males and females were housed together in a 1:1 o for mating. They were allowed a three week period for mating and were rated once evidence of mating was seen. One litter was produced in each ration. After weaning of the first generation, thirty males and thirty les were selected for the second parental generation. The remaining male ntal animals were sacrificed on days 133-134 of the study. Animals cted for the second generation were exposed to test diets for 12 weeks r to mating. Mating was conducted in the same manner as for the first ration. Parental males were sacrificed on day 138 of the study and ntal females on days 138, 139, and 152 after weaning of their litters.

Atrazine had no effect on the reproductive parameters studied; however, weights at postnatal day 21, second generation were statistically ificantly lower than those of the control group at 50 and 500 ppm. Body nts, body weight gain and food consumption were statistically ificantly decreased for parental animals, males and females, throughout study at the HDT. In addition, a statistically significant increase in tive testes weights was seen in both generations. Therefore, the oductive NOEL and LEL are 10 and 50 ppm (0.5 and 2.5 mg/kg/day), ectively, and the Parental NOEL and LEL are 50 and 500 ppm (2.5 and 25 g/day), respectively.

A.3. UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

100. An uncertainty factor of 100 was used to account for the inter- and species differences.

1.

A.4. ADDITIONAL COMMENTS (ORAL RfD)

Considered for Establishing the RfD

Year Feeding - dog: NOEL=15 ppm (0.48 mg/kg/day); LEL=150 ppm (4.97 g/kg/day) (statistically significant decreased P-II waves in females at y 175 of study and cardiac toxicity seen in two male dogs): core grade nimum (Ciba-Geigy Corp., 1987a)

2) 2-Generation Reproduction - rat: Reproductive NOEL=10 ppm (0.5 mg/kg/day); Reproductive LEL=50 ppm (2.5 mg/kg/day) (decreased body weights of pups of the second generation on postnatal day 21); Parental NOEL=50 ppm (2.5 mg/kg/day); Parental LEL=500 ppm (25 mg/kg/day) (decreased body weights, body weight gain, and food consumption in both parental males and females throughout the study; In addition, the increase in relative testes weights seen in parental males could be treatment-related since it was seen in both generations); core grade minimum (Ciba-Geigy Corp., Agricultural Division, 1987b)

3) 2-Year Feeding/Oncogenicity - rat: Systemic NOEL=70 ppm (3.5 mg/kg/day); Systemic LEL=500 ppm (25 mg/kg/day) (reduced body weights and food consumption); core grade minimum (Ciba-Geigy Corp., 1986)

4) Teratology - rat: Maternal NOEL=10 mg/kg/day; Materanl LEL=70 mg/kg/day (reduced body weight gain in first half of gestation; High mortality at 700 mg/kg/day); Developmental NOEL=10 mg/kg/day; Developmental LEL=70 mg/kg/day (delayed ossification); core grade minimum (Ciba-Geigy Corp., 1984a)

5) Teratology - rabbit: Maternal NOEL=1 mg/kg/day; Maternal LEL=5 mg/kg/day (reduced body weight gain and reduced food consumption); Developmental NOEL=5 mg/kg/day; Developmental LEL=75 mg/kg/day (HDT; increased resorptions, decreased fetal weights of male and female pups, delayed ossification of appendages); core grade minimum (Ciba-Geigy Corp., 1984b)

Other Data Reviewed:

1) Oncogenicity - mouse: NOEL=300 ppm (45 mg/kg/day); LEL=225 mg/kg/day (decreases of 23.5% and 11.0% in mean body weight gain found at 91 weeks in male and female mice, respectively and increase in the incidence of cardiac thrombi in female mice); core grade guideline (Ciba-Geigy Corp., Agricultural Division, 1987c)

2) 2-Year Feeding - dog: Systemic NOEL=15 ppm [14.1 ppm (0.35 mg/kg/day); analytical value]; Systemic LEL=150 ppm [141 ppm (3.5 mg/kg/day); analytical value] (increased heart and liver weights in females; effects at 1500 ppm included reduced food intake, decreased body weight, reduced hemoglobin and hematocrit values); core grade supplementary (Ciba-Geigy Corp., 1964)

3) 3-Generation Reproduction - rat: Systemic NOEL>100 ppm (5 mg/kg/day) (HDT); Reproductive NOEL>100 ppm (5 mg/kg/day) (HDT); core grade supplementary (Ciba-Geigy Corp., 1966)

4) Teratology - rat: Maternal NOEL=100 mg/kg/day; Maternal LEL=500 mg/kg/day (weight loss); Fetotoxic NOEL=100 mg/kg/day; Fetotoxic LEL=500 mg/kg/day (fetal resorptions, weight loss); core grade minimum (Ciba-Geigy, Corp., 1971)

Data Gap(s): None

___I.A.5. CONFIDENCE IN THE ORAL RfD

Study: High
Data Base: High
RfD: High

The critical study is of good quality and is given a high confidence rating. Additional studies are supportive and of good quality and therefore he data base is given a high confidence rating. High confidence in the RfD ollows.

.I.A.6. EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

istration Standard
istration Files

cy RfD Work Group Review: 05/20/87, 06/22/88

fication Date: 06/22/88

.7. EPA CONTACTS (ORAL RfD)

Ghali / OPP -- (703)557-7490 / FTS 557-7490

gler / OPP -- (703)557-7491 / FTS 557-7491

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

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EPA: 68-02-4225
DYNAMAC No. 2308
March 20, 1987

006937

DATA EVALUATION RECORD

ATRAZINE

Chronic Oral Toxicity/Oncogenicity Study in Rats

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 3-20-87

EPA: 68-02-4225
DYNAMAC No. 2308
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006937

DATA EVALUATION RECORD

ATRAZINE

Chronic Oral Toxicity/Oncogenicity Study in Rats

REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: 3-20-87

Nicolas P. Hajjar, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Nicolas P. Hajjar
Date: 3/20-87

APPROVED BY:

I. Cecil Felkner, Ph.D.
Oncogenicity/Chronic Toxicity
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 3-26-87

Henry Spencer, Ph.D.
EPA Reviewer

Signature: Henry Spencer
Date: 5/5/87

Albin Kocialski, Ph.D.
EPA Section Head (VII)

Signature: AKC
Date: 5/5/87

006937

DATA EVALUATION REPORT

006937

TOX. CHEM. NO.: 63
MRID NO.: 00141874

STUDY TYPE: Chronic oral toxicity/oncogenicity study in rats.

ACCESSION NUMBER: 262714-262727.

TEST MATERIAL: Atrazine technical.

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylaminotriazine.

STUDY NUMBER(S): 410-1102.

SPONSOR: CIBA GEIGY Corp., Greensboro, NC.

TESTING FACILITY: American Biogenics Corporation, Decatur, IL.

TITLE OF REPORT: Twenty-four month combined chronic oral toxicity and oncogenicity study in rats utilizing atrazine technical.

AUTHOR(S): Mayhew, D. A., Taylor, G. D., Smith, S. H., and Banas, D. A.

REPORT ISSUED: April 29, 1986.

CONCLUSIONS:

Under the conditions of the study, atrazine was oncogenic in CD-1 Sprague-Dawley rats; an increase in carcinomas of the mammary gland was observed in females fed 70, 500, or 1000 ppm atrazine for 2 years. There was also an increase in the incidence of fibroadenomas/adenomas (1000 ppm) as well as all mammary tumors in females receiving 500 and 1000 ppm when compared to controls. There was a decrease in mean body weights of males and females receiving 500 and 1000 ppm. Survival was decreased in high-dose females but increased in high-dose males. Red cell parameters (hemoglobin, hematocrit, and red cell count) were decreased in high-dose females but not in males. The serum glucose level was decreased in high-dose females at 3, 6, and 12 months and serum triglyceride levels tended to be decreased in high-dose males throughout the study; however, the toxicologic importance of the clinical chemistry findings is unclear. There were decreases in organ-to-body weight ratios in high-dose animals, which were probably the result of body weight decreases. Hyperplastic changes in high-dose males (mammary gland, bladder, and prostate) and females (myeloid tissue of bone marrow and transitional epithelium of the kidney) were of questionable toxicologic importance. There was an increase in retinal degeneration and in centrolobular necrosis of the liver in high-dose females and an increase in degeneration of the rectus femoris muscle in high-dose males and females when compared to controls. Based on decreased body weight gain, the LOEL for chronic toxicity in males and females is 500 ppm and the NOEL is 70 ppm.

Core Classification: Core Minimum. The study can be upgraded to Core Guideline if individual animal disposition data can be provided.

A. MATERIALS:

1. Test Compound: Atrazine technical; description: a white powder, batch No. FL0821575; stated purity: 98.9 percent--contaminants were not described.

2. Test Animals: Species: rat; strain: Sprague-Dawley [Crl:COBS CD (SD) BR]; age: 30 days at receipt and 37 to 38 days at initiation; weight (mean): 110-111 g for females and 145-146 g for males; source: Charles River Breeding Laboratories, Portage, MI. Animals were acclimated for 7 to 8 days.

B. STUDY DESIGN:

1. Animal Assignment: Animals were assigned randomly to the following groups using a computer-randomized program:

	Test Group	Dose in Diet (ppm)	Chronic Toxicity Subgroup		Oncogenicity Subgroup		Interim Sacrifices 12 mos.		Interim Sacrifices 13 mos.[b]	
			M	F	M	F	M	F	M	F
1	Control	0	20	20	70	70	10	10	9	10
2	Low (LDT)	10	20	20	50	50	—	—	—	—
3	Mid low	70	20	20	50	50	—	—	—	—
4	Mid (MDT)	500	20	20	50	50	—	—	—	—
5	High (HDT)	1000	20	20	70	70	10	10	10	10

[a]The chronic toxicity animals were primarily for clinical laboratory analyses.

[b]These rats received control diets for 1 month prior to sacrifice.

2. Diet Preparation: Diets were prepared weekly and presented to animals within 3 days of preparation. Homogeneity and stability were assayed prior to study initiation and diets were analyzed monthly for concentration of test compound. The test material was also assayed monthly to determine its stability.

Results: The test material was stable throughout the study; the mean (±2 SD) purity was 95.9±2.3 (range, 93.3-98.0) percent. Homogeneity was acceptable and the concentration of the test material recovered from the diets after 2 weeks of storage at ambient temperature was greater than 92 percent. Mean concentrations of the test material in diets for 25 intervals of analysis and at 95 percent confidence limits (±2 SD) were 9.90±0.79, 70.2±3.0, 503±19, and 999±35 ppm for nominal levels of 10, 70, 500, and 1000 ppm, respectively.

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3. Animals received Purina Certified Rodent Chow No. 5002 (with or without test compound) and water ad libitum. Animals were caged individually in temperature- and humidity-controlled rooms where the air was filtered through HEPA and charcoal.

4. Statistics: Body weight, food consumption, clinical pathology, and organ weight data were analyzed by ANOVA and significant differences examined by Tukey's (equal populations) or Scheffe's (unequal populations) tests for multiple comparisons. Non-parametric data were analyzed by the Kruskal-Wallis test. Survival was examined by the Cox-Tarone test using life tables as well as by the nonparametric-ranked score test of Gehan and Breslow for trend and heterogeneity. Graphical evaluation of survival was determined by Kaplan-Meir product-limit estimates.

   Nonneoplastic incidences were evaluated by the Cochran-Armitage trend test and the Fisher-Irwin exact test with the Bonferroni adjustment for significance level ($p = 0.05/4, 0.0125$).

   Some neoplastic incidences were analyzed by the method of Dinse and Lagakos[1] because of observed intercurrent mortality differences. This method allows delineating the effects of dose x age on prevalence; continuity corrections were applied. Where appropriate, adenomas and carcinomas were combined (and in some cases hyperplasia) for analysis; carcinomas alone were also evaluated. Analysis of pituitary tumors in females combined adenoma and carcinoma, and both prevalence and life-table analyses were performed.

5. A quality assurance statement was signed and dated April 29, 1986.

C. METHODS AND RESULTS:

1. Observations: All animals were observed twice daily for mortality, morbidity, and overt toxic signs; individual animals were examined weekly and palpated for tissue masses.

   Results: There was an increase in the incidence of tissue masses in females receiving 70, 500, or 1000 ppm (Table 1). Irritability was noted at an increased incidence in males receiving 500 ppm (n=22) and 1000 ppm (n=26) when compared to controls (n=13). Other signs of toxicity were those commonly seen in rats and were at a similar incidence in both dosed and control groups.

   Mortality and percent survival at selected intervals are summarized in Table 2. In males, survival was increased in a dose-related manner ($p < 0.003$, using the Cox Tarone test) and was significantly higher in males receiving 1000 ppm when compared to controls ($p = 0.0055$, using pairwise comparison). In contrast,

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[1] Dinse, G. E. and Lagakos, S. W. (1983) Regression analysis of tumor prevalence data, J. R. Stat. Soc. Ser. 32:236-248.

TABLE 1. Palpable Tissue Mass Observations in Rats Fed Atrazine for 2 Years

Location	Number of Animals with Mass									
	Dose Level (ppm)									
	Males					Females				
	0	10	70	500	1000	0	10	70	500	1000
Abdomen	19	21	17	19	22	33	36	40	52	58
Axilla	0	1	0	0	0	2	3	6	9	9
Chest	2	0	1	4	2	10	14	18	27	32
Perianal	0	0	0	2	0	4	2	4	20	15
Perineum	5	1	1	2	7	9	14	23	20	35
Back	6	4	5	8	7	2	2	3	2	12
Side	2	3	0	7	8	19	21	31	38	47

TABLE 2. Cumulative Mortality and Percent Survival[a] at Selected Intervals in Rats Fed Atrazine for 2 Years

Dose Group (ppm)	Mortality (Percent Survival) at Week		
	52	78	Termination
		Males	
0	2 (97)	13 (83)	40 (44)
10	3 (96)	11 (84)	37 (47)
70	3 (96)	10 (86)	31 (56)
500	1 (99)	10 (86)	30 (57)
1000	1 (99)	6 (94)	23 (67)
		Females	
0	2 (97)	11 (84)	35 (50)
10	5 (93)	16 (77)	39 (44)
70	0(100)	12 (83)	40 (43)
500	2 (97)	13 (81)	44 (37)
1000	2 (97)	17 (76)	52 (26)

[a]Percent Survival was based on 70 rats/sex/group except for control males (n=71).

survival in females was decreased in a dose-related manner, (p value for a negative trend = 0.0016) and was significantly lower (p = 0.0042) in high-dose females when compared to controls.

2. Body Weight: Rats were weighed weekly for 13 weeks and monthly thereafter. Body weights at scheduled sacrifices were determined for fasted animals.

Results: Representative body weight data are summarized in Table 3. Mean body weights were significantly depressed in both males and females receiving 500 and 1000 ppm with the exception of the mean weights of males in the last 2 months of the study. The 24-month weight gain in the high-dose animals was 76 percent of control for the males and 64.5 percent of control for the females. In the recovery groups, the weight gain for month 13 for males previously receiving 1000 ppm was 63±14.6 g, compared to 20±17.9 g for controls ($p \leq 0.01$), and for females previously receiving 1000 ppm it was 56±31.6 g, compared to 16±11.9 g for controls ($p \leq 0.01$). However, the mean weight at 13 months in these males was still significantly ($p \leq 0.01$) lower than controls.

3. Food Consumption and Compound Intake: Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.

Results: Representative food consumption data are summarized in Table 4. Food consumption values were significantly decreased in mid- and high-dose males for the first 13 months, for the mid-dose females for the first 12 weeks of the study, and for the high-dose females for the first 6 months. No significant differences in food consumption were noted in recovery males or females during month 13, although there were significant increases in weight gain in male and female groups that had previously received 1000 ppm. Food efficiency was similar in dosed and control groups.

4. Ophthalmology: Ophthalmologic examinations were performed on control and high-dose rats prior to study initiation, prior to sacrifice at 12 and 13 months, and prior to terminal sacrifice.

Results: There were no adverse findings at the interim sacrifices. The changes seen at terminal sacrifice, mild keratitis, neovascularity, or cataracts, were considered due to use of powdered food, to orbital bleeding, or to aging. These finding occurred at a similar frequency in control and high-dose males and females.

5. Blood Parameters: Blood was collected before treatment and at 3-month intervals for hematology and clinical chemistry analysis from all surviving animals in the chronic toxicity subgroups (initially 20/sex/group). It was also collected from 10/sex in the control and high-dose groups scheduled for sacrifice at 12 and 13 months. The CHECKED (X) parameters were examined.

TABLE 3. Mean Body Weights at Selected Intervals in Rats Fed Atrazine for 2 Years

Dose Level (ppm)	Mean Body Weight (g±SD) at Week						
	0	1	13	26	52	78	104
				Males			
0	146±18.2	212±21.3	532±44.2	637±63.8	727±91.4	767±110.7	704±124.7
10	146±18.2	213±19.7	541±47.2	646±71.2	737±98.4	783±139.3	739±140.5
70	146±17.6	209±19.0	516±41.6	617±61.0	713±82.6	779± 97.2	742± 97.7
500	145±18.0	199±19.3**	467±39.9**	548±50.6**	635±70.3**	678± 95.6**	646±119.6
1000	145±17.2	189±19.9**	436±37.6**	508±42.3**	574±52.1**	610± 61.8**	572± 97.0**
				Females			
0	110±11.8	154±14.1	283±27.2	330±36.0	408±59.3	467± 95.2	496±105.7
10	111±12.1	155±13.8	282±25.2	328±39.7	404±67.2	475± 95.1	477±132.6
70	111±11.3	153±13.2	275±26.0	324±38.1	405±67.2	471± 89.4	480±106.8
500	111±12.4	145±13.3**	252±20.3**	289±26.6**	342±42.6**	371± 73.7**	402±106.8*
1000	111±12.2	139±11.2**	239±21.4**	271±27.6**	308±36.1**	341± 59.1**	361±81.1**

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

TABLE 4. Food Consumption Data at Selected Intervals in Rats Fed Atrazine for 2 Years

Dose Level (ppm)	Daily Mean Food Consumption (g/rat) at Week					
	1	13	26	52	78	104
			Males			
0	22.2	27.2	27.1	26.7	24.5	22.2
10	22.1	26.6	26.9	27.2	25.9	23.7
70	21.4	26.1	26.2	26.6	24.1	22.9
500	19.7**	24.1**	24.6**	25.3	23.0	22.1
1000	17.7**	22.7**	23.4**	23.8**	23.2	21.0
			Females			
0	17.5	18.1	19.7	20.7	18.8	18.1
10	17.8	18.3	19.3	20.5	20.2	17.4
70	17.9	18.0	19.6	20.0	19.8	17.3
500	16.1**	17.0	19.1	20.0	17.9	18.1
1000	14.4**	16.7**	18.4*	19.8	17.8	16.5

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

a. Hematology

X	Hematocrit (HCT)†	X	Leukocyte differential count
X	Hemoglobin (HGB)†	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)†	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)†	X	Mean corpuscular volume (MCV)
X	Platelet count†	X	Heinz bodies
X	Coagulation time		

Results: In females receiving 1000 ppm, significantly lower mean RBC, HGB, and HCT were noted at 6, 12, and 18 months when compared to controls (Table 5). The values were somewhat depressed in high-dose females at 24 months, however, only four females were used for clinical studies. RBC, HGB, and HCT were also decreased in the 10 females receiving 1000 ppm and scheduled for sacrifice at 12 months. The values approached control levels at 13 months in the 1000-ppm recovery group. All values for parameters in dosed males were similar to those in the control groups with the exception of an increased mean platelet count at 6 months in rats receiving 1000 ppm. Increased platelet counts were also seen at 6 and 12 months in females receiving 1000 ppm.

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium†	X	Albumin†
X	Chloride†		Blood creatinine†
	Magnesium†	X	Blood urea nitrogen† (BUN)
X	Phosphorus†	X	Cholesterol†
X	Potassium†	X	Globulins and A/G ratio
X	Sodium†	X	Glucose†
	Enzymes	X	Total bilirubin†
X	Alkaline phosphatase	X	Total protein†
	Cholinesterase (RBC and serum)	X	Triglycerides
	Creatinine phosphokinase†		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)†		
X	Serum aspartate aminotransferase (also SGOT)†		
X	Gamma glutamyl transpeptidase (GGT)		
X	Creatine phosphokinase		

Results: The level of serum triglycerides in high-dose males was, in general, lower than control values throughout the study (Table 6); however, the decrease was significant only at 6 months. In groups scheduled for the 12-month sacrifice, the level in high-dose males (103.10±47.81 mg/dL) was significantly lower ($p \leq 0.01$) than in control males (228.30±95.44 mg/dL). At the

†Recommended by Subdivision F (October 1982) guidelines for chronic studies.

TABLE 5. Selected Hematology Data on Female Rats Fed Atrazine for 2 Years

Parameter/ Monthly Interval	Dietary Level (ppm) 0	500	1000
Erythrocytes (10^6/mm^3)			
3	7.74±0.30 (20)[a]	7.66±0.41 (20)	7.40±0.27 (20)
6	7.43±0.39 (19)	7.17±0.33 (20)	6.84±0.41**(20)
12	6.74±0.39 (19)	6.14±0.81 (18)	5.95±1.13* (18)
18	7.72±1.00 (17)	7.04±0.63 (15)	6.29±1.09**(12)
24	7.20±1.21 (8)	6.14±1.43 (6)	6.36±0.90 (4)
Hemoglobin (g/dL)			
3	16.33±0.59 (20)	16.23±0.66 (20)	15.76±0.59 (20)
6	15.70±0.77 (20)	15.29±0.65 (20)	14.66±0.84**(20)
12	13.55±0.80 (19)	12.63±0.98 (18)	12.15±2.19* (18)
18	14.18±1.64 (17)	13.19±1.22 (15)	12.13±1.86* (12)
24	13.60±1.62 (8)	12.12±2.01 (6)	12.30±1.21 (4)
Hematocrit (%)			
3	41.86±1.44 (20)	41.52±0.84 (20)	40.26±1.58 (20)
6	39.46±1.89 (20)	38.50±1.90 (20)	36.91±2.18**(20)
12	42.04±3.03 (19)	39.08±3.73 (18)	37.53±6.73* (18)
18	42.98±4.87 (17)	39.79±3.40 (15)	36.46±5.41* (12)
24	42.06±5.11 (8)	37.68±6.54 (6)	38.08±3.35 (4)

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

[a]The numbers of animals included in calculation of the mean ±SD are given in parentheses.

TABLE 6. Serum Triglyceride Levels (±SD) in Male Rats Fed Atrazine for 2 Years

Dose Level (ppm)	Serum Triglyceride Level (mg/dL) at Month				
	3	6	12	18	24
0	98.37± 64.16	139.26± 88.35	221.05±145.16	210.12±192.78	141.82± 72.35
10	105.79± 62.29	147.74± 98.68	241.16±103.89	225.75±124.14	120.88± 54.49
70	89.55± 55.64	123.85± 84.87	251.32±149.51	232.13±143.48	211.31±116.93
500	62.21± 14.42	87.21± 66.94	171.89± 58.32	171.89± 69.51	141.73± 64.90
1000	53.00± 14.36	56.55± 18.12*	110.45± 43.08	133.00± 59.88	107.85± 62.45

*Significantly different from control value ($p \leq 0.05$).

13-month sacrifice, the triglyceride level was similar in control males and those that had previously received 1000 ppm atrazine. In females, glucose levels were decreased ($p < 0.01$) in the high-dose group at 3, 6, and 12 months when compared to controls (Table 7). Other changes in clinical chemistry parameters occurred sporadically in high-dose animals and were not considered compound related since there were no patterns consistent with dose or time.

6. Urinalyses: Urine was collected from fasted animals (chronic toxicity groups) at 3, 6, 12, and 18 months and prior to termination. It was also collected from animals of the control and high-dose groups prior to sacrifice at 12 and 13 months. The CHECKED (X) parameters were examined.

X	Appearance†	X	Glucose†
X	Volume†	X	Ketones†
X	Specific gravity†	X	Bilirubin†
X	pH	X	Blood†
X	Sediment (microscopic)†	X	Nitrate
X	Protein†	X	Urobilinogen

Results: Urinalysis data in dosed groups were comparable to control data and within the normal ranges.

7. Sacrifice and Pathology: All animals that died or were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue		Aorta†	XX	Brain† (3 levels)
X	Salivary glands†	X	Heart†	X	Peripheral nerves (sciate)†
X	Esophagus†	X	Bone marrow†	X	Spinal cord (3 levels)
X	Stomach†	X	Lymph nodes†	X	Pituitary†
	(3 areas)	X	Spleen†	X	Eyes†
X	Duodenum†	X	Thymus†		Glandular
X	Jejunum†		Urogenital	XX	Adrenals†
X	Ileum†	XX	Kidneys†		Lacrimal gland
X	Cecum†	X	Urinary bladder†	X	Mammary gland†
X	Colon†	XX	Testes†	X	Parathyroids†
X	Rectum†	XX	Epididymides	XX	Thyroids†
XX	Liver†	XX	Prostate		Other
	(2 sections)	X	Seminal vesicle	X	Bone (femur)
	Gall bladder†	XX	Ovaries	X	and bone marrow†
X	Pancreas†	X	Uterus/cervix	X	Skeletal muscle†
	Respiratory			X	Skin
X	Trachea†			X	Harderian glands
X	Lung/bronchi†			X	All gross lesions and masses

†Recommended by Subdivision F (October 1982) guidelines for chronic studies.

TABLE 7. Serum Glucose Levels (mg/dL) in Female Rats Fed Atrazine for 2 Years

Interval (Month)	Dose Level (ppm)	
	0	1000
3	135.95± 14.91[a]	117.25± 12.16*
6	148.79± 15.31	129.79± 20.40*
12	127.16± 20.52	103.50± 22.74*
12[b]	121.80± 14.82	99.70± 19.32*
18	112.18± 29.75	104.00± 22.28
24	112.06±118.40	88.25± 20.01

*Significantly different from control value ($p \leq 0.05$).

[a]Mean ± standard deviation.

[b]Rats (10) from the oncogenicity group scheduled for interim sacrifice.

Results:

a. Organ Weights: The absolute weight of liver and kidney in high-dose males sacrificed at 12 months was significantly ($p \leq 0.05$) lower than controls. The mean weight of the liver was 14.71±2.81 g for the high-dose group and 19.70±3.46 g for the controls; the mean weight of the kidneys was 3.67±0.40 g for high-dose males, compared to 4.39±0.58 g for controls.

At 24 months, the mean absolute weights of liver and kidney in high-dose males were lower than those of controls, but the decrease was not statistically significant. There were no other changes in absolute organ weights of males and females.

There were several increases in organ-to-body weight ratios in high-dose animals that were significant ($p \leq 0.05$) when compared to controls but they were not accompanied by changes in absolute organ weights. These changes were the result of decreased body weights. At 12 months, the organ-to-body weight ratios for brain and kidney were increased in high-dose males; at 13 months, the ratios for brain, kidney, and testes were increased and at 24 months the ratios for brain and testes were increased in high-dose males. In high-dose females, the organ-to-body weight ratios for adrenal, brain, kidney, and liver were increased at 12 and 24 months. There was an increase in the ovary-to-body weight ratio at 13 months in the recovery group that had previously received 1000 ppm.

b. Gross Pathology: For most organs there were very few gross abnormalities noted at necropsy. There were no notable abnormalities at the 12- and 13-month interim sacrifices when high-dose rats were compared to controls. In the main study, there was an increased incidence of abnormal pelvic contents in the kidneys of high-dose males (10/65) when compared to controls (4/67). In females, there were some increases in the incidence of masses in the abdominal, thoracic, and axillary regions in rats receiving 70, 500, and 1000 ppm when compared to controls. Correlation of gross and microscopic findings indicated that most of the masses were mammary tumors. Masses found on weekly palpations were correlated with gross finding for all individual males and all high-dose females. An excellent correlation was found (see Discussion).

c. Microscopic Pathology:

1. Nonneoplastic: A summary of selected nonneoplastic lesions is presented in Table 8. Several proliferative lesions occurred with increased frequency in atrazine-dosed rats. Acinar hyperplasia of the mammary gland and epithelial hyperplasia of the prostate were increased in males receiving 1000 ppm when compared to controls. In females receiving 500 or 1000 ppm there was an increased

TABLE 8. Nonneoplastic Lesions in Rats Fed Atrazine for 2 Years[a]

Organ/Finding	Dietary Level (ppm)									
	Males					Females				
	0	10	70	500	1000	0	10	70	500	1000
Mammary gland	(58)[b]	(59)	(61)	(64)	(65)	(66)	(64)	(68)	(65)	(64)
Acinar hyperplasia	7	1	5	7	21**T	0	0	0	0	0
Bone marrow—femur	(65)	(63)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(64)
Myeloid hyperplasia	23	30	21	21	27	25	23	24	38**	52**T
Bone marrow—sternum	(65)	(65)	(67)	(67)	(66)	(68)	(65)	(69)	(65)	(64)
Myeloid hyperplasia	23	28	21	27	26	21	21	20	33*	46**T
Spleen	(65)	(65)	(67)	(67)	(67)	(67)	(65)	(69)	(65)	(65)
Extramedullary hematopoiesis	6	10	14	9	9	12	14	18	22*	28**T
Kidney	(65)	(65)	(67)	(67)	(67)	(68)	(65)	(68)	(65)	(65)
Pelvic calculi	15	16	11	17	31**T	57	52	57	55	60
Micro calculi	3	5	3	7	8	3	0	0	3	2
Hyperplasia, transitional epithelium	9	8	12	13	13	17	10	5**	19	31**
Urinary bladder	(65)	(65)	(67)	(67)	(67)	(67)	(65)	(69)	(65)	(64)
Hyperplasia, transitional epithelium	4	2	5	3	5	4	0	1	3	10*
Prostate	(65)	(63)	(66)	(67)	(66)					
Epithelial hyperplasia	12	16	11	17	29**T					
Muscle—rectus femoris	(64)	(65)	(67)	(66)	(67)	(67)	(65)	(69)	(64)	(64)
Degeneration	6	7	7	10	28**T	5	4	9	8	13*T
Eye	(65)	(65)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(65)
Retinal degeneration	2	2	5	5	7	12	9	13	16	22*T
Liver	(65)	(65)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(65)
Centrolobular necrosis	6	3	1	2	2	3	3	1	4	12**

[a] Includes animals at terminal sacrifice and those that died or were sacrificed moribund from month 13 to study termination.

[b] The number of tissues examined is given in parentheses.

*Significantly different from control incidence ($p \leq 0.05$).

**Significantly different from control incidence ($p \leq 0.01$).

T Positive dose-related trend ($p \leq 0.01$).

incidence of myeloid hyperplasia in the bone marrow of both the femur and sternum. It was reported that the bone marrow changes, as well as an increase in extramedullary hematopoiesis in the spleen, were sequellae related to mammary fibroadenomas and adenocarcinomas. The myeloid hyperplasia was characterized by a decrease in the number of fat cells in the marrow and an increase in hematopoietic tissue, particularly cells of the granulocytic series. In females receiving 1000 ppm, there was also an increased incidence of hyperplasia of the transitional epithelium of both the kidney ($p < 0.01$) and urinary bladder ($p > 0.05$). The incidence of calculi in the renal pelvis in high-dose males was increased compared to controls. Muscle degeneration (femoral muscle) was found in both high-dose males and females. Retinal degeneration was increased in both dosed males and females; the incidence being significantly ($p \leq 0.05$) higher in the high-dose females than in controls. In high-dose females there was an increase in coagulative centrolobular necrosis in the liver. There was a slight increase in chronic pododermatitis in females receiving 500 and 1000 ppm (16-17%) when compared to controls (1%) but the incidence was much lower than in all groups of males including controls (30-39%).

2. Neoplastic: Table 9 summarizes the neoplastic lesions found in rats that died from month 13-24 or were sacrificed at termination. There was an increased incidence of mammary adenocarcinoma in females receiving 70, 500, or 1000 ppm atrazine and an increase in fibroadenoma in high-dose females. Several females had multiple mammary tumors. There was also an increase in adenocarcinoma in high-dose females at the 12- and 13-month sacrifices (Table 10). Statistical analysis by the report authors included all animals on study and used life-table analysis and pairwise comparison with the Cox-Tarone test and Gehan-Breslow test. These results are included in Table 10. There were statistically significant increases ($p < 0.05$) in carcinomas for females receiving 70, 500, and 1000 ppm atrazine, in adenomas and fibroadenomas for females receiving 1000 ppm, and in total mammary tumors in females receiving 500 and 1000 ppm. There were significant ($p < 0.00005$) positive dose trends for all three categories (sarcomas, fibroadenomas plus adenomas, and all mammary tumors).

D. STUDY AUTHORS' CONCLUSIONS:

Atrazine was oncogenic in female CD Sprague-Dawley rats. There were increased incidences of mammary carcinomas at 70, 500, and 1000 ppm, all mammary tumors at 500 and 1000 ppm, and of mammary adenoma and fibroadenoma at 1000 ppm. Survival was significantly higher in males

TABLE 9. Neoplastic Lesions in Rats Fed Atrazine for 2 Years[a]

Organ/Neoplasm	Dietary Level (ppm)									
	Males					Females				
	0	10	70	500	1000	0	10	70	500	1000
Brain	(65)[b]	(65)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(65)
Chromophobe carcinoma	3	1	1	0	1	2	1	3	8	7
Astrocytoma	1	0	0	0	2	0	0	0	0	0
Adrenal	(65)	(65)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(65)
Pheochromocytoma	11	9	4	5	5	1	2	2	3	4
Cortical adenoma	1	3	0	0	0	5	5	6	4	3
Pituitary	(59)	(64)	(67)	(66)	(62)	(68)	(63)	(68)	(65)	(63)
Chromophobe adenoma	22	22	29	24	17	47	41	49	47	35
Chromophobe carcinoma	5	7	6	7	4	9	6	9	14	13
Thyroid	(63)	(65)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(65)
C-cell adenoma	12	9	8	21	9	8	10	6	5	5
C-cell carcinoma	2	4	2	3	3	0	0	1	1	0
Follicular cell adenoma	5	4	1	2	3	1	1	1	0	0
Kidney	(65)	(65)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(65)
Liposarcoma	0	0	5	0	2	0	0	0	0	0
Liver	(65)	(65)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(65)
Hepatocellular carcinoma	4	5	0	1	2	0	1	1	0	0
Hepatocellular adenoma	0	0	1	2	3	1	1	2	1	0
Pancreas	(64)	(65)	(67)	(67)	(67)	(67)	(65)	(69)	(65)	(65)
Islet cell adenoma	4	5	4	2	3	3	0	3	1	0
Islet cell carcinoma	1	1	3	2	0	5	1	2	0	0
Testis	(65)	(65)	(67)	(67)	(67)					
Interstitial cell tumor	1	3	2	2	7					
Uterus						(67)	(65)	(69)	(62)	(65)
Endometrial stromal polyp						4	6	3	0	3

(Continued)

[a]This tabulation includes animals sacrificed at termination or found dead or sacrificed moribund between 13 months and study termination. Statistical notations are not included in this table since the authors' analysis included animals sacrificed at 12 and 13 months.

[b]The number of tissues examined is in parentheses.

TABLE 9. Neoplastic Lesions in Rats Fed Atrazine for 2 Years[a] (Continued)

Organ/Neoplasm	Dietary Level (ppm)									
	Males					Females				
	0	10	70	500	1000	0	10	70	500	1000
Mammary gland	(58)	(59)	(61)	(64)	(65)	(66)	(64)	(68)	(65)	(64)
Adenocarcinoma	0	1	0	0	1	15	15	26	27	35
Fibroadenoma	1	1	1	1	0	29	29	35	38	42
Adenoma	0	0	0	1	1	1	0	1	1	2
Carcinosarcoma	0	0	0	0	0	0	0	0	0	2
Skin	(4)	(6)	(3)	(7)	(6)					
Keratoacanthoma	1	3	0	2	2	0	0	2	0	1
Lipoma	2	0	0	0	0	1	0	0	0	0
Fibroma	0	0	0	0	3	0	0	0	0	0
Trichoepithelioma	0	0	0	2	0	0	0	0	0	0
Head										
Zymbal gland carcinoma	4	2	0	3	1	0	0	1	0	0

(Concluded)

[a] This tabulation includes animals sacrificed at termination or found dead or sacrificed moribund between 13 months and study termination. Statistical notations are not included in this table since the authors' analysis included animals sacrificed at 12 and 13 months.

[b] The number of tissues examined is in parentheses.

TABLE 10. Mammary Tumors in Female Rats Fed Atrazine for 2 Years

	Dose Level (ppm)				
	0	10	70	500	1000
12-month sacrifice/deaths and moribund sacrifices at 0-13 months					
No. tissues examined	12	5	1	5	15
Adenocarcinoma	0	1	1	0	3
Fibroadenoma	0	0	1	1	1
13-month sacrifice					
No. tissues examined	10	--	--	--	10
Adenocarcinoma	0	--	--	--	5
Fibroadenoma	0	--	--	--	2
Terminal sacrifice/deaths and moribund sacrifices at 13-24 months					
No. tissues examined	66	64	68	65	65[a]
No. rats--adenocarcinoma and carcinosarcoma	15	15	26	27	37
No. of adenocarcinomas	17	22	42	48	64
No. rats--fibroadenoma	29	29	35	38	42
No. of fibroadenomas	37	46	48	81	69
Mammary tumor-bearing rats	35	39	47	47	56[a]
All animals on study					
No. of tissues	88	69	69	70	89
Carcinomas	15	16	27	27	45
Adenomas and fibroadenomas	29	29	36	39	46
All tumors	35	40	48	48	65
p values[b]					
Carcinomas--Cox-Tarone			0.0454	0.0071	<0.00005
--Gehan Breslow			0.0290	0.0016	<0.00005
Adenomas and fibroadenomas--Cox-Tarone				0.0685	0.0004
All tumors--Cox-Tarone				0.0071	<0.00005
--Gehan-Breslow				0.0050	<0.00005

[a] Values differ from those in final report by 1.

[b] Life-table analysis, pairwise comparison.

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ATRAZINE 080803

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Reviewed by: Sanford W. Bigelow, Ph.D.
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D.
Section VI, Toxicology Branch (TS-769C)

Judith W. Hauswirth 3/26/88

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: oncogenicity - mouse (83-2)

CASWELL NO: 63

ACCESSION NUMBER:

MRID NO.: 404313-02

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: 842120

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: Division of Toxicology/Pathology, Ciba-Geigy Corp., Summit, NJ 07901

TITLE OF REPORT: Atrazine - technical: 91-week oral carcinogenicity study in mice.

AUTHORS: J.R. Hazelette, Ph.D. and J.D. Green, Ph.D.

REPORT ISSUED: October 30, 1987

CONCLUSIONS: Atrazine was not oncogenic to the CD-1 strain of mouse under the conditions of this assay.

NOEL = 300 ppm (45.0 mg/kg)
LEL = 1500 ppm (225.0 mg/kg) based upon the effects found in male and female mice. The NOEL and the LEL were determined on the following bases. The LEL of 1500 ppm was based upon decreases of 23.5% and 11.0% in mean body weight gain found at 91 weeks in male and female mice, respectively. Also, an increase in the incidence of cardiac thrombi was found in female mice in the 1500 ppm exposure group. None of the above effects were found at 300 ppm, thus the NOEL for atrazine in mice was set at 300 ppm.

At the highest exposure level, 3000 ppm, atrazine exposure in both sexes of mice caused:

1) a decrease in the mean body weight gain at 12 and 91 weeks,

2) a decrease in food consumption rates,

3) an increase in the incidence of cardiac thrombi,

4) a decrease in erythrocyte count, hemoglobin concentration and hematocrit, and

in female mice only, atrazine exposure caused:

1) an increase in mortality,

2) a decrease in mean brain and kidney weights, and

3) decreased percentages of neutrophils and lymphocytes.

Classification: core-guideline: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §83-2 have been satisfied.

II. MATERIALS:

A. Test Compound: atrazine

Description: atrazine, technical-grade
Batch #: 841802
Purity: The purity of atrazine used in this study was not given.

B. Test Animals:

Species: Mouse
Strain: CD-1 [Crl: CD1 (ICR) BR]
Age: about 5 weeks
Weight (mean, in grams): females: 21.0 ± <20%
(at week 0) males: 26.8 ± <20%
Source: Charles River Laboratories, Kingston, NY

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study

Test Group	Dose in diet (ppm)	Main Study 91 weeks male	Main Study 91 weeks female	Least number of treatment weeks
1 Control	-	59	60	91
2 Low1 (LDT1)	10	60	59	91
3 Low2 (LDT2)	300	60	60	91
4 Mid1 (MDT1)	1500	60	60	91
5 High (HDT)	3000	58	60	91

Upon arrival from Charles River Laboratories, all mice were quarantined for 2 weeks for observation prior to initiation of atrazine exposure. Atrazine feeding started October 31, 1984 and ended August 22, 1986.

B. Diet Preparation:

The diet containing atrazine was prepared within 2 weeks before initial atrazine exposure and thereafter, about every 3 weeks. Every lot of feed containing atrazine was used within 3 weeks of preparation. The feed was stored at room temperature, and on several occasions, at refrigerated temperatures. The feed was analyzed for concentration and/or homogeneity on weeks 5, 9, 13, 17, 21, 29, 33, 37, 41, 45, 49, 60, 68, 76, 84, 92 and 94. This analysis was performed in the Toxicology/Pathology Administration and Technical Operations Section (of Ciba-Geigy in Summit, NJ) prior to use.

Analytical results: These admixtures were reported to be stable for at least 40 days at room temperature. Analytical results state that storage at room temperature caused less than 10% variation in the stability, homogeneity or concentration of atrazine in the laboratory chow.

The drinking water (tap water) was analyzed periodically according to the standard operating procedure of the Safety Evaluation Facility and was found to contain no detectable levels of contaminants.

Feeding schedule: Animals received food (called Certified Purina Rodent Chow #5002) and water ad libitum throughout the 91 week study.

C. Statistics:

The following statistical procedures were utilized in analyzing the numerical data:

The Barlett's test was conducted for determining homogeneity in variances (presence of a normal distribution) between treatment groups. If the variance was found to be similar between groups by the above tests, Dunnett's tests were conducted to compare values of the control and treatment groups.

When outliers (or heterogeneous variances between groups) were identified, supplemental statistical analyses were performed. Examples of these supplemental statistical analyses were: (1) the use of an appropriate transformation of the data or (2) nonparametric tests. In addition, several test results that are known not to be distributed normally were analyzed with the use of nonparametric tests.

Concerning the pathology data sets, if their sample size was found adequate, these data were analyzed for each sex by the Fisher's exact test. Tumor incidences were analyzed by a time-adjusted analysis by Peto's method. Statistical differences for survival curves between treatment groups for each sex were examined by the use of the following statistical methods: (1) the generalized Wilcoxon test for equality, (2) the Mantel-Cox logrank test equality and test for linear trend, (3) nonparametric tests and (4) Kaplan-Meier estimates.

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector. According to the statement, the study was audited sixteen (16) times during the course of the study.

IV. METHODS AND RESULTS:

A. Clinical Observations:

Animals were inspected twice daily for mortality and once daily for general appearance, behavior and excreta.

Viral screens were performed on 6 male and 6 female mice taken randomly from the colony 2 weeks before atrazine feeding began. The presence of the following viruses were checked: minute virus, pneumonia virus, reovirus (type 3), hepatitis virus, K virus, murine encephalomyelitis virus, Sendai virus, lymphocytic choriomeningitis virus, adenovirus, ectromelia virus, polyoma virus and mycoplasma pulmonis.

Toxicity/mortality (survival) results: A total of 301 of the 596 mice used in the study died. For female mice fed 3000 ppm atrazine, there was a statistically significant decrease in survival whereas for males, atrazine exposure had no statistically significant effect on survival.

Table 2
Summary of Mortality
(taken from p. 41)

Sex:	Males					Females				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
# of mice:	59[a]	60	60	60	58[b]	60	59[a]	60	60	60
Reason for sacrifice:										
Found dead:	20	18	16	18	17	27	32	27	29	39
Sacrificed moribund:	4	9[c]	8	3	6	7	4	7	4	6
Terminal sacrifice:	35	33	36	39	35	26	23	26	27	15
% survival at term:	59	55	60	65	60	43	39	43	45	25[d]

[a] Two mice were deleted due to misidentification.
[b] Two mice in group 5 (3000 ppm) were mis-sexed, and therefore their data results were deleted from reporting.
[c] One mouse in Group 2 (10 ppm) escaped from its cage and was sacrificed.
[d] $p < 0.05$, generated from a survival analysis with the use of Mantel-Cox Logrank test.

Clinical Results: There were no treatment-related clinical signs observed during the study. Clinical signs most frequently observed were: lesions, alopecia, scabs, perineal stains, fur stains and dermatitis in all groups of mice. No treatment-related changes in the incidence of palpable masses occurred during the study.

B. Body weight:

All animals were weighed weekly for weeks 1-12, biweekly during weeks 14-25, and at 4 week intervals thereafter.

Results: Dose-related reductions were observed at weeks 12 and 91 in mean body weight gain (either % decrease or % gain) in both sexes of mice who were exposed to chow containing 1500 ppm or 3000 ppm atrazine. Table 3 shows the changes in mean body weight gain at weeks 12 and 91 in mice fed atrazine. At week 12 males exhibited decreases of 14.2% and 10.4% in mean body weight gain in the 10 ppm and 300 ppm exposure groups, respectively. This effect appears to be transient in nature because it is not observed at 91 weeks at the same magnitude.

Table 3
Mean Body Weight Gain Changes in Mice Fed Atrazine
(taken from Table 8.3)

Dose (ppm)	Week 12 Gain (g)	Week 12 % Decrease[a] (Increase)	Week 12 % Gain[b]	Week 91 Gain (g)	Week 91 % Decrease[a] (increase)	Week 91 % Gain[b]
			Males			
0	10.6	NA	39.1	11.5	NA	43.7
10	9.1	14.2	37.1	13.3	(15.6)	49.4
300	9.5	10.4	36.1	10.4	9.6	39.5
1500	7.1	33.0	26.7[d]	8.8	23.5	32.9[c]
3000	6.3	40.6	23.8[d]	8.7	24.4	33.1[c]
			Females			
0	9.1	NA	43.7	13.6	NA	68.8
10	9.3	(2.2)	44.5	13.0	4.4	64.6
300	8.5	6.6	40.5	12.6	7.4	64.1
1500	7.8	14.3	37.9[c]	12.1	11.0	61.7
3000	8.0	12.1	37.6[c]	7.0	48.5	33.3[d]

[a] The reviewer calculated body weight gain by the following formula:

$$\% \text{ Decrease (increase)} = 100 - \frac{\text{Mean body weight gain (test group)}}{\text{Mean body weight gain (control)}} \times 100$$

where: Mean body weight gain = body weight (g) for wk 12 (or wk 91) - body weight (g) for wk 0

[b] The authors of the study calculated % body weight gain by the following formula:

$$\% \text{ Gain} = \frac{\text{Body weight (week 12 or 91)}}{\text{Body weight (week 0)}} \times 100$$

[c] $p = \leq 0.05$ and [d] $p = \leq 0.01$, significantly different from control group when compared by the use of the two-tailed Dunnett t-test performed on the raw data.
NA = Not applicable

C. Food consumption and compound intake:

Food consumption was determined in all animals on a weekly basis during weeks 1-12, biweekly during weeks 14-25 and at 4 week intervals thereafter. Mean daily diet consumption were calculated from these data. The intake of atrazine was calculated and is reported below. Efficiency and atrazine intake were calculated from the consumption and body weight gain data.

Water consumption was measured in all animals on weeks 1, 2, 52, 53, 90 and 91. [Subdivision F (§83-2, section 7 part vi) states that water consumption should be monitored weekly during the first 13 weeks of a study and then at 4 week intervals thereafter (p. 121).]

Food consumption results: Treatment-related reductions in mean food consumption were observed in Group 4 males (1500 ppm) and Group 5 males and females (3000 ppm). Statistically significant reductions in mean water intake were noted primarily in mice fed 1500 or 3000 ppm atrazine. Reductions in mean food consumption correlated with similar reductions in mean body weight and mean body weight gain. These reduction were sporadic (occurred only in certain weeks during the study) and were not related to the dose of atrazine. No statistically significant reductions in mean food consumption were seen in mice fed chow containing 10 ppm atrazine.

Table 4
Dietary Intake of Atrazine
(taken from p. 19)

		Dietary Concentration	Mean Daily Dose (mg/kg)[a]	Range (mg/kg/day)
Males:				
Group #	2	10	1.4	1.2 - 2.0
	3	300	38.4	35.7 - 58.3
	4	1500	194.0	184.3 - 293.3
	5	3000	385.7	364.3 - 541.6
Females:				
Group #	2	10	1.6	1.4 - 2.3
	3	300	47.9	41.1 - 73.1
	4	1500	246.9	215.9 - 363.3
	5	3000	482.7	420.8 - 660.6

[a] The group mean daily dose was calculated for each study week as follows:

$$\text{Group Mean Daily Dose (mg/kg)} = \frac{\text{Group Mean Food Consumption (g/mouse/day)} \times \text{Atrazine Conc. (mg/kg)}}{\text{Mean Group Mid-Period Body Weight (g)}}$$

Conclusion: On the basis of a daily dose of mg/kg, female mice fed 300, 1500, or 3000 ppm atrazine received about a 25% higher daily dose of atrazine than male mice in the corresponding exposure group.

D. Ophthalmological examination:

Ophthalmological examinations were performed prior to the study on all male and all female rats on weeks 26, 52, 78 and 90.

Results and conclusions: No treatment-related ophthalmic changes were observed during this study. Corneal opacities and lenticular cataracts were the most frequent observations and occurred with similar incidence in both control and treated groups of mice (see table below). Most animals with ocular changes noted early in the study (i.e., examined at weeks 26 or 52) had no ocular changes when examined at weeks 78 and 90.

Table 5
Summary Incidence of Ocular Findings at 90 Weeks
(taken from p. 2709)

Sex:	Males					Females				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
# of mice:	38	36	37	43	37	28	25	27	31	15
Ocular Findings:										
Cornea: opacity	14	9	10	17	12	9	9	4	10	2
Lens: catract	22	17	20	17	24	23	18	24	25	15
Adnexa:										
blepharitis	2	3	3	2	3					
Iris:										
ectopic pupil	1		1	1	2					
Phthisis bulbi	1									

E. Hematology:

Blood was collected from all animals on days 362, 544 and 639 for hematology and clinical analysis from all animals. Blood smears were obtained during weeks 52 and 78 from the first 20 animals for each sex in the 0 ppm and 3000 ppm atrazine groups. In addition, all animals who died or who were sacrificed in moribund conditon had blood smears taken. The CHECKED (X) parameters were examined.

|X|	Hematocrit (HCT)*	|X|	Leukocyte differential count*
|X|	Hemoglobin (Hb)*	| |	Mean corpuscular Hb (MCH)
|X|	Leukocyte count (WBC)*	|X|	Mean corpuscular Hb conc.(MCHC)
|X|	Erythrocyte count (RBC)*	|X|	Mean corpuscular volume (MCV)
| |	Platelet count*+	| |	Reticulocyte count
| |	Plateletcrit	| |	Mean platelet volume
| |	Platelet dist. width	| |	Red cell dist. width
| |	Blood clotting msrmts.		
| |	(Thromboplastin time)		
| |	(Clotting time)		
| |	(Prothrombin time)		

* Required for subchronic and chronic studies
\+ Not required for oncogenicity studies

Results and conclusions: At the termination of the study, statistically significant reductions in mean erythroid variables (erythrocyte count, hematocrit and hemoglobin) were observed in Groups 4 (1500 ppm) and 5 (3000 ppm) males and Group 5 females. The authors concluded that these erythroid effects were secondary to decreased body weight, food consumption and/or water consumption. These results are summarized in Table 6.

Other hematological effects were observed. Group 5 females (3000 ppm) had reduced mean neutrophil percentage and elevated lymphocyte percentage when compared to control mice (Table 6). These elevated blood cell levels may have been caused by by illness, although the authors did not fully elaborate on these results.

A few male and female blood samples in those mice who survived to terminal necropsy were not analyzed, regardless, the number of samples only amounted to 1 per group (compare Tables 2 and 9).

Table 6
Selected Hematological Parameters in Mice at 639 Days
(taken from Table 8.7)

Group #:	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000
		# of mice examined			
males:	34	33	35	39	34
females:	26[c]	23	26[c]	26	15
Parameter:					
			Males		
RBC	7.68	7.47	7.48	6.69[b]	6.33[b]
Hb	14.71	14.02	14.07	12.86[a]	12.52[b]
HCT (%)	45.24	43.21	43.66	39.7[a]	38.62[b]
			Females		
RBC	6.64	7.34	6.36	6.29	5.54[a]
Hb	13.25	14.33	12.62	12.58	11.22[a]
HCT (%)	41.04	43.96	39.04	38.62	34.80[a]
Neuts %[c]	39.52	37.22	40.84	49.23	56.40[a]
Lymphs %[c]	58.32	60.61	57.92	48.54	42.33[a]

[a] p = <0.05, [b] p = <0.01, significantly different from control group when compared by the use of the two-tailed Dunnett t-test performed on the raw data.
[c] = For neutrophil % and lymphocyte % tests, 25 female control mice and 25 female mice in the 300 ppm exposure group were examined.

F. Sacrifice, Gross Pathology and Histopathology:

All animals were fasted overnight prior to terminal necropsy. The 301 animals that died in the course of the study and those mice who were sacrificed on schedule were examined for gross pathological and histological changes. Terminal necropsies began August 1, 1986 and ended August 22, 1986 on weeks 92-95 of the study. Necropsies were also performed on the animals who had died during the course of the study. Microscopic examinations were performed on all specificed tissues and gross lesions from all animals in each group, regardless whether the animal was found dead, sacrificed moribund, or after scheduled necropsy.

The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed to determine the organ weight.

	Digestive system
X	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
X	Gall bladder*#
X	Pancreas*

	Respiratory
X	Trachea*#
X	Lung*
	Nose^
X	Pharynx^
X	Larynx^

	Cardiovascular
X	Aorta*
X	Heart*
X	Bone marrow*#
X	Lymph nodes*
X	Spleen
X	Thymus*

	Urogenital
XX	Kidneys*+
X	Bladder*
XX	Testes*+
X	Epididymides
X	Prostate
X	Seminal vesicle
X	Ovaries*+
X	Uterus*
	Cervix
	Fallopian tubes
X	Vagina

	Neurological
XX	Brain*+
X	Periph. nerve (sciatic)*#
X	Spinal cord (3 levels)*#
X	Pituitary*
X	Eyes (optic n.)*#

	Glandular
XX	Adrenal gland*
	Exorbital lacrimal gland#
X	Mammary gland*#
X	Parathyroids*++
X	Thyroids*++

	Other tissues
	Bone (femur)*#
X	Skeletal muscle(thigh)*#
X	Skin*#
X	All gross lesions and masses*

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated by signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

1. Organ weight: Organ weights were determined for the liver, kidneys, testes, brain and adrenals in all animals in all exposure groups at weeks 92-95 during terminal necropsy. Organ weights were not recorded for animals found dead or sacrificed moribund. Only 26 of the 36 surviving females had their livers weighed whereas 34 of 35 surviving males had their livers weighed, and therefore, not all of the mice who were fed atrazine had their organs examined.

   Organ weight results: Few organ weight changes were observed in this study. Mice fed 3000 ppm exhibited decreased mean weight in the following tissues: brain (females and males) and kidneys (females). Organ weight increases were found in mean brain to body weight ratio in females. Table 7 shows the organ weights of the brain, adrenal gland, kidney, liver and testes as well as the organ weight ration to whole body weight.

Table 7
Selected Organ Weights and Weight Ratios at 91 Weeks
(% body weight ratios in parentheses)
(taken from Table 8.8)

Group #:	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000
Organ (in grams):					
Males					
Brain	0.55 (1.55)	0.50 (1.45)	0.50 (1.55)	0.49 (1.55)	0.48[b] (1.52)
Adrenal	0.007 (0.02)	0.008 (0.02)	0.007 (0.02)	0.007 (0.02)	0.007 (0.02)
Kidney	0.65 (1.96)	0.67 (1.93)	0.64 (1.95)	0.62 (1.96)	0.60 (1.90)
Liver	1.61 (4.81)	1.62 (4.75)	1.53 (4.69)	1.54 (4.85)	1.53 (4.83)
Testes	0.32 (0.96)	0.32 (0.94)	0.33 (1.01)	0.31 (1.00)	0.29 (0.94)
Females					
Brain	0.52 (1.73)	0.53 (1.78)	0.52 (1.76)	0.51 (1.83)	0.49[a] (2.01)[b]
Adrenal	0.01 (0.04)	0.01 (0.03)	0.01 (0.03)	0.01 (0.03)	0.01 (0.05)
Kidney	0.49 (1.61)	0.48 (1.62)	0.47 (1.60)	0.44 (1.59)	0.41[b] (1.70)
Liver	1.60 (5.26)	1.48 (4.93)	1.49 (5.02)	1.54 (5.50)	1.41 (5.68)

[a] $p = <0.05$ [b] $p = <0.01$, significantly differnet from control group when compared by the use of the two-tailed Dunnett t-test performed on the raw data.

2. Gross pathology results: Several gross observations were noted in the mice fed higher levels (i.e., 1500 ppm and/or 3000 ppm) of atrazine. These observations were: enlarged atrium (or atria) of the heart, tan-colored lesions of the heart and pallid color of the kidney(s). The observation of enlarged atria of the heart appears to be dose-related although it occurs at a low incidence. The incidences of these gross lesions are illustrated in the Table 8 below.

Table 8
Summary Incidence of Gross Lesions Observed in this Study
(taken from Table 9.6.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
Total # of mice:	59	60	60	58	58	60	59	60	60	60
Organ or Site:										
Heart, l. atrium:										
enlarged	1			4	4			1	4	8
Lesion, tan				2	2				3	3
Heart, r. atrium:										
enlarged	1			1	2				1	13
Kidney, pallid color	2		3	2		3	4	3	10	3

3. Histopathology results:

a) Non-neoplastic lesions: As seen after the terminal necropsy, dose-related cardiac thrombi (primarily in the atria) were seen in male mice receiving 1500 ppm and female mice fed 3000 ppm atrazine. As shown in Table 9, cardiac thrombi were observed primarily in those animals who had died or were killed in the course of the study.

The authors attributed the majority of the unscheduled deaths to spontaneously-occurring renal amyloidosis (p. 24). However, the incidence of cardiac thrombi in mice with unscheduled deaths and who were sacrificed moribund is statistically significant from control mice (this group of mice is referred by the authors as "early deaths" -- this term is adopted in this review). Statistically significance was not found in any group of mice regarding the incidence of renal amyloidosis in any group of mice. Table 9 shows that the incidence of cardiac thrombi in female mice treated with 1500 ppm or 3000 ppm was higher in the "early death" group of female mice than the corresponding female mice who survived to terminal necropsy.

Other statistically significant amyloid lesions occurred in exposed groups but were termed "sporadic" by the authors. The following amyloid lesions were observed:

1) A statistically significant increase in incidence of amyloidosis of the liver and the adrenal gland were found in female mice fed 300 ppm atrazine.

2) Likewise, thyroid amyloidosis was observed the "early death" group of female mice who ate chow containing 10 ppm atrazine.

3) Lymph node amyloidosis was observed in the "early death" group of female mice who were fed 1500 ppm atrazine.

Table 9
Summary Incidence of Cardiac Thrombi Observed in this Study
(taken from p. 2749)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early death" group	3/24	5/27	3/24	7/21	9/23[a]	3/34	4/36	1/34	11/33[b]	24/45[a]
Mice surviving to terminal sacrifice	0/35	1/33	0/36	0/39	0/35	0/26	0/23	1/26	0/27	2/15
All mice	3/59	6/60	3/60	7/60	9/58	3/60	4/59	2/60	11/60[a]	26/60[c]

[a] = $p < 0.05$, [b] = $p < 0.01$, [c] = $p < 0.001$, significantly different from control (by Fisher's exact test).

Table 10
Summary Incidence of Renal Amyloid Lesions Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early death" group	9/24	10/27	6/24	8/21	6/23	18/34	22/36	23/34	20/32	25/45
Mice surviving to terminal sacrifice	5/35	2/33	4/36	3/39	8/35	8/26	5/23	7/26	5/27	0/15
All mice	16/59	9/60	14/60	14/60	18/58	28/60	31/59	31/60	25/60	29/60

Table 11
Summary Incidence of Adrenal Amyloid Lesions Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early deaths" group	8/24	8/27	6/24	11/20	9/22	18/34	24/35	24/33	20/32	30/44
Mice surviving to terminal sacrifice	3/32	1/32	2/35	0/37	8/32	5/26	2/23	9/25	6/27	0/15
All mice	11/56	9/59	8/59	11/57	17/54	23/60	26/58	33/58[a]	26/59	30/59

[a] = $p < 0.05$, significantly different from control (by Fisher's exact test).

Table 12
Summary Incidence of Hepatic Amyloid Lesions Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early deaths" group	7/24	7/27	4/24	10/20	9/23	17/34	23/36	23/34	19/33	29/45
Mice surviving to terminal sacrifice	2/35	1/33	2/35	0/39	4/34	4/26	2/23	6/25	5/27	0/15
All mice	8/59	7/60	5/60	10/59	10/58	20/60	25/59	30/60[a]	22/60	29/60

[a] = $p < 0.05$, significantly different from control (by Fisher's exact test).

Table 13
Summary Incidence of Lymph Node Amyloid Lesions Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early deaths" group	3/22	0/25	0/23	0/16	3/21	5/34	8/32	10/32	11/31[a]	8/41
Mice surviving to terminal sacrifice	1/35	1/31	2/35	0/36	1/33	3/26	3/23	3/26	3/26	0/14
All mice	4/57	1/56	2/58	0/52	4/54	8/60	11/55	13/58	14/57	8/55

[a] = $p < 0.05$, significantly different from control (by Fisher's exact test).

Table 14
Summary Incidence of Thyroid Gland Amyloid Lesions Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early death" group	8/24	8/25	5/23	9/21	10/22	17/34	25/34[a]	23/34	20/33	29/45
Mice surviving to terminal sacrifice	2/35	1/33	2/35	0/39	4/34	4/26	2/23	6/25	5/27	0/15
All mice	10/59	9/58	7/58	9/60	14/56	21/60	27/57	29/59	25/60	29/60

[a] = $p < 0.05$, significantly different from control (by Fisher's exact test).

b) Neoplastic lesions: Overall, atrazine exposure did not cause a dose-related increased incidence of neoplasms in these mice in this study.

Histological evaluation of palpable masses were performed. Of the palpable masses examined, 3 female mice were found to have developed mammary adenocarcinomas (one mouse in the control group and 2 mice fed chow containing 3000 ppm atrazine). One female in the 10 ppm exposure group developed a fibroma and one female in the 300 ppm group developed malignant lymphoma. In male mice, two developed fibrosarcoma in the 10 ppm exposure group. One male mouse in the group fed 1500 ppm atrazine was determined to have a hemangiosarcoma (a malignancy formed by the proliferation of endothelial and fibroblastic tissue). These neoplasms were found after histological examination of these palpable masses in these mice; some of these tumors are listed in the tumor incidence tables below.

As shown on Table 15, in male mice fed 10 ppm atrazine, a statistically significant increase in the incidence of hepatocellular adenomas was observed, yet no statistically significant increase in incidence of this type of tumor was observed in groups of mice fed higher levels of atrazine (i.e., 300 ppm, 1500 ppm or 3000 ppm). This effect is not dose-related.

No statistically significant increases in incidence were found for the following types of neoplasms: mammary adenocarcinomas, adrenal adenomas, pulmonary adenomas and malignant lymphomas. The incidences for these tumors are listed in the tables below.

Table 15
Summary Incidence of Hepatocellular Adenomas Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early death" group	0/24	5/27[a]	0/24	1/20	0/23	0/34	0/36	0/34	0/33	0/45
Mice surviving to terminal sacrifice	10/35	8/33	6/36	3/39	1/35	1/26	0/23	0/26	0/27	0/13
All mice	10/59	13/60	6/60	4/59	1/58	1/60	0/59	0/60	0/60	0/60

[a] = $p < 0.05$, significantly different from control (by Fisher's exact test).

Table 16
Summary Incidence of Mammary Adenocarcinomas Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early death" group	0/13	0/9	0/13	0/14	0/9	1/33	0/35	0/33	0/33	2/44
Mice surviving to terminal sacrifice	0/18	0/12	0/19	0/22	0/20	0/26	0/23	1/25	0/27	0/15
All mice	0/31	0/21	0/32	0/36	0/29	1/59	0/59	1/58	0/60	2, 59

Table 17
Summary Incidence of Adrenal Adenomas Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early deaths" group[a]	0/24	0/27	0/24	0/20	0/22	0/34	0/35	0/33	0/32	0/44
Mice surviving to terminal sacrifice	3/32	0/32	3/35	4/37	3/32	0/26	0/23	0/25	0/27	0/15
All mice	3/56	0/59	3/59	4/57	3/54	0/60	0/58	0/58	0/59	0/59

[a] = The numerator of these incidence values in this row were calculated by subtracting the tumor incidence in those mice who survived until terminal sacrifice from all of the mice studied (e.g., for Group 1 males, 3/56 - 3/32 = 0/24)

Table 18
Summary Incidence of Pulmonary Adenomas Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early deaths" group	1/24	1/27	1/24	0/21	1/23	1/34	1/36	1/34	1/33	1/45
Mice surviving to terminal sacrifice	3/35	3/33	3/36	5/39	6/35	0/26	0/23	1/26	2/27	1/15
All mice	4/59	4/60	4/60	5/60	7/58	1/60	1/59	2/60	3/60	2/60

Table 19
Summary Incidence of Malignant Lymphoma Observed in this Study
(taken from Tables 9.6.1.1, 9.6.1.2, 9.6.1.3)

Sex:	Male Mice					Female Mice				
Group #:	1	2	3	4	5	1	2	3	4	5
Dose (ppm):	0	10	300	1500	3000	0	10	300	1500	3000
"Early deaths" group	2/24	3/27	5/24	0/21	1/23	6/34	5/36	5/34	3/33	3/45
Mice surviving to terminal sacrifice	2/35	5/33	4/36	3/39	3/35	13/26	9/23	11/26	9/27	4/15
All mice	4/59	8/60	9/60	3/60	4/58	19/60	14/59	16/60	12/60	7/60

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receiving 1000 ppm and significantly lower in females receiving 1000 ppm than in controls. There was a significant decrease in body weight throughout the study for males and females in the 500- and 1000-ppm groups. Food consumption was decreased in males receiving 500 and 1000 ppm for the first year of the study, for females receiving 1000 ppm for the first 6 months, and for females receiving 500 ppm for the first 3 months. Red cell parameters were decreased in females receiving 1000 ppm but not in males. Glucose was decreased in 1000 ppm females at 3, 6, and 12 months and triglycerides were decreased in 1000-ppm males at 3 and 6 months. Nonneoplastic findings were limited to animals receiving 1000 ppm. An increase in mammary acinar cell hyperplasia, kidney calculi, and epithelial hyperplasia of the prostate in high-dose males may be associated with increased survival in this group. Retinal degeneration and centrolobular necrosis of the liver were increased in high-dose females; there was an increase in degeneration of the rectus femoris muscle in high-dose males and females. Increased occurrence of transitional cell hyperplasia in the kidney and bladder of high-dose females was of questionable significance. The NOEL for chronic toxicity was considered to be 70 ppm atrazine in the diet.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

In agreement with the report authors, we assess that atrazine was carcinogenic in female CD rats, causing an increase in mammary adenocarcinomas at dietary levels of 70, 500, and 1000 ppm. The report authors found a high level of statistical significance and a positive dose-related trend ($p < 0.00005$) with both the Cox-Tarone test and the Gehan-Breslow test using life-table analysis. The concurrent control incidence of mammary adenocarcinoma (17.05%) was somewhat higher than the mean value for four other studies (9.5 percent, range 3.8-18.9 percent) performed by the testing laboratory. The biological importance of the significant increase in mammary adenoma plus fibroadenoma in high-dose females ($p = 0.004$) is not as clear as that of the adenocarcinomas. The concurrent control incidence of fibroadenoma (29/88, 33 percent) is slightly lower than the historical incidence for the laboratory (mean 41.4 percent, range of four studies 36.3-47.8 percent).

We assess that the study authors correctly interpreted that the increase of interstitial cell tumors of the testes in high-dose males (7/67) when compared to controls (1/65) was probably related to increased survival in the males, which resulted in more late-appearing tumors. The incidence of the finding in final sacrifice males was 6/47 in the high-dose group and 1/31 in controls; in those that died between 13-24 months, the incidence was 1/20 in the high-dose group and 0/34 in controls. Historical control incidence for interstitial cell tumors in terminal sacrifice animals was 8 percent (range, 0-19 percent). The incidence in high-dose males was 12 percent in the current study.

Time to mammary tumor could not be calculated since the individual animal disposition tabulations were not provided. However, we noted that 8/25 high-dose females (compared to 0/22 controls) had mammary adenocarcinomas by 13 months (Table 10).

Weekly palpation data were available for all animals. These data were checked against the gross and histologic findings to determine if all in-life masses were followed through with a gross finding and a histologic diagnosis. The data for all males were checked as well as the data for high-dose females. Masses that disappeared were checked for the first and last day of observation.

In the high-dose females, more than 90 percent of all masses observed in-life persisted until sacrifice or death. There were none that disappeared in the last 2 months of the study. The followup at gross and histologic examination was excellent. For female 8364 there was no section for a mass and for female 8429 the masses were lost; female 8183 had a small mass that was not a tumor and female 8330 had an abdominal mass but no tumor at gross or histologic examination. All other masses had a histologic diagnosis.

In males, there were several masses that were transient. Cell masses that persisted were confirmed by gross or histologic examination. The following lists in-life masses that disappeared in males:

Dose (ppm)	No. animals	No. animals with masses	No. masses
0	90	20	32
10	70	19	36
70	70	17	43
500	70	25	42
1000	90	27	56

In four control males and in two high-dose males masses not seen in-life were found on gross examination and confirmed histologically. None of the males that died by month 13 and none that were sacrificed at 12 months had mammary tumors. Mammary tumors in males were as follows: control, fibroadenoma (8051); 10 ppm, adenocarcinoma (7805), fibroadenoma (7906); 70 ppm, fibroadenoma (7951); 500 ppm, adenoma (7701), fibroadenoma (8017); 1000 ppm, adenocarcinoma (8063), fibroma (7896). Other masses were diagnosed as abcesses, galactocoeles, lipomas, fibromas, papillomas, histiocytic sarcomas, zymbal gland carcinomas, etc. In two males receiving 1000 ppm (8030 and 3818), late appearing masses were not found on gross examination. It is our assessment that the gross and histologic followup of in-life masses was excellent.

There was an increase in several hyperplastic lesions in rats receiving 1000 ppm atrazine. The increase in hyperplasia of the transitional epithelium in kidney and urinary bladder in high-dose females is probably compound related. The increase in acinar hyperplasia of the mammary glands and in epithelial hyperplasia of the prostate in high-dose males could be compound related and/or the result of increased survival in this group. Other lesions noted in high-dose animals such as muscle degeneration (males and females), retinal degeneration (females), and pelvic calculi in the kidney (males) are normally occurring lesions of aging.

The decrease in red cell parameters in high-dose females and increase in myeloid hyperplasia and increased extramedullary hematopoiesis in females receiving 500 or 1000 ppm atrazine may be a consequence of the development of mammary tumors.

The increases in organ-to-body weight ratios noted in the study are primarily due to decreased body weights. The effects of triglycerides and glucose may also be related to the weight loss. When all chronic toxicity parameters are considered it is reasonable to set a LOEL at 500 ppm and a NOEL at 70 ppm.

The study was well conducted and adequately reported. Once individual animal disposition data are provided, the study can be classified Core Guideline.

Reviewed by: Judith W. Hauswirth, Ph.D., Section Head Judith W Hauswirth 4/28/88
Section VI, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 2-Generation Reproduction Study (83-4) TOX. CHEM. NO.: 63

MRID NO.: 404313-03

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NO.: 852063

SPONSOR: Ciba-Geigy Corp., Agricultural Division, Greensboro, NC 27419

TESTING FACILITY: Research Department, Pharmaceuticals Divison, Ciba-Geigy Corporation, Summit, NJ 07901

AUTHORS: J Mainiero, M Youreneff, MLA Giknis and ET Yau

REPORT ISSUED: November 17, 1987

CONCLUSION: Parental NOEL = 50 ppm
Parental LEL = 500 ppm based upon decreased body weights, body weight gain, and food consumption in both parental males and females throughout the study. In addition, the increase in relative testes weights seen in parental males could be treatment-related since it was seen in both generations.

Reproductive NOEL = 10 ppm
Reproductive LEL = 50 ppm based upon decreased body weights of pups of the second generation on postnatal day 21.

CORE CLASSIFICATION: Core - Minimum

A. MATERIALS:

1. Test compound: Atrazine, Technical. Batch FL 841802. White powder. Purity not specified but stated to be on record at Ciba-Geigy Corporation, Greensboro, NC.

2. Test animals: Species: rat; Strain: Charles River (CRCD, VAF/PLUS) from Charles River Laboratory, Ltd., Kingston, NY; Age: 37 days; Weight: males, 177-219g and females, 140-176g; animals were quarantined for one week.

6. STUDY DESIGN:

1. Animal assignment: According to the report, "One hundred twenty male and one hundred twenty female rats from the acclimation colony were randomly assigned a TEROS® temporary animal identification number and at the same time were randomly distributed into 4 treatment groups..." Permanent numbers were assigned when the animals were found acceptable for the study.

2. Experimental design: Male rats were placed on the control and test diets at 47 days of age and females at 48 days of age. They were maintained on these diets for a period of 10 weeks prior to mating. Males and females were housed together in a 1:1 ratio for mating. They were allowed a three week period for mating and were separated once evidence of mating was seen. One litter was produced in each generation. After weaning of the last litter of the first generation, thirty males and thirty females were selected for the second parental generation. The remaining male parental animals were sacrificed on days 113-114 of the study. The remaining female parental animals were sacrificed on days 133-134 of the study.

Animals selected for the second parental generation were exposed to test diets for 12 weeks prior to mating. Mating was conducted in the same manner as for the first generation. Parental males were sacrificed on day 138 of the study and parental females on days 138, 139 and 152 after weaning of their litters.

3. Test diet: Atrazine was mixed with Purina #5002 Certified Rodent Chow. The concentrations used were 0, 10, 50 and 500 ppm. Diets containing 10 to 3000 ppm atrazine were found to be stable at room temperature for at least 40 days. Periodic homogeneity analyses were performed and atrazine concentrations were found to be 93-105% of the expected values.

4. Statistics: Statistical methods can be found in Appendix 1 (Section 2.13 of the report).

5. A signed quality assurance statement was included with the study report.

C. METHODS, RESULTS, AND DISCUSSION:

1. Parental animals:

a. Observations: Animals were observed once daily for signs of toxicity and twice daily for mortality. No treatment-related clinical signs were seen in either parental generation. Alopecia and sore(s)/scab(s) were commonly seen in all groups including the controls.

At the levels tested, atrazine had no effect on mortality in either parental generation.

b. Body weights: Body weights were determined weekly and at termination for males. For females, body weights were recorded weekly during the premating phase, on days 0, 7, 14 and 20 of gestation and on days 0, 4, 7, 14, and 21 of lactation. Selected body weight data can be found summarized in the following table for both parental generations.

Selected Parental Body Weight Data

Mean Body Weights (g)					
	Day				
Dose (mg/kg)	0	21	49	70	Terminal
			Males, F_0		
0	198.4	339.0	448.8	500.9	566.3
10	198.1	338.5	449.8	508.9	577.8
50	197.6	337.4	447.8	501.2	567.8
500	198.0	309.3*	396.0*	440.7*	484.8*
			Males, F_1		
0	167.8	337.6	478.4	541.1	642.3
10	160.8	329.8	471.6	528.5	626.2
50	160.6	325.2	462.6	529.6	627.4
500	146.7*	294.7*	408.9*	459.5*	540.1*
			Females, F_0		
0	158.0	220.8	261.2	281.7	
10	154.8	215.2	258.4	280.0	
50	155.2	209.8	254.5	269.9	
500	154.2	197.5*	231.6*	243.5*	
			Females, F_1		
0	141.7	212.0	262.8	287.8	
10	138.7	216.4	272.1	296.1	
50	140.1	212.4	264.5	290.4	
500	127.9*	193.7*	232.7*	251.8*	

Body Weights (Cont'd.)

	Females, F_0 (Gestation)		Females, F_0 (Lactation)		
	0	20	0	14	21
0	289.5	407.0	330.5	354.2	341.3
10	285.3	415.9	323.5	348.2	333.9
50	281.5	410.0	320.8	344.9	331.3
500	250.6*	376.6*	288.3*	319.1*	314.7*
	Females, F_1 (Gestation)		Females, F_1 (Lactation)		
0	302.0	408.4	329.8	347.5	333.7
10	296.5	413.4	334.7	344.5	335.3
50	305.3	418.1	341.4	346.7	333.3
500	260.8*	370.3*	297.6*	316.9*	315.2*

* $p<0.05$

Body weights were statistically significamtly lower for both males and females fed the diet containing 500 ppm atrazine (HDT) throughout the study. Body weight gains were also statistically significantly depressed at the HDT. At the mid dose (50 ppm) sporadic statistically significant decreases in body weight gain were noted. These changes are not considered to be related to treatment since they were occasional and very sporadic.

c. Food consumption: Food consumption was determined weekly for males and females during the premating period and on days 0, 7, 14, and 20 of gestation for the females.

Food consumption was statistically significantly reduced for males and females during the premating period for both parental generations and for F_1 females on days 0-7 of gestation.

d. Sacrifice and pathology: All parental animals were subjected to gross pathological examination. The testes and ovaries were weighed. The following tissues were collected for microscopic examination:

vagina	cervix	ovaries
testes	epididymides	seminal vesicles
prostate	pituitary	coagualtion gland
gross lesions		

Tissues from the control and high dose group were examined microscopically as well as <u>all</u> gross lesions.

1) Organ weights: There were no treatment-related effects on ovarian weights. Relative but not absolute testes weights were statistically significantly increased at the HDT

in parental males of both generations. The study authors attributed this change to decreased body weight gain at this dosage level.

2) Gross necropsy: No treatment-related effects were seen in either generation.

3) Histopathology: No treatment-related effects were seen in either generation.

2. Reproductive effects:

a. Pup weights: Mean pup weights per litter were recorded on lactation days 0, 4, 7, 14, and 21. Selected overall mean pup weights for each dosage group and each generationn are shown in the following table.

Mean Pup Weights (g)

F_1 Generation	Day				
Dosage Group (ppm)	0	4 (pre-culling)	7	14	21
0	6.42	9.11	14.43	31.00	49.87
10	5.99*	8.10*	12.95*	28.31*	45.09*
50	6.17	8.56	13.54	29.87	47.23
500	6.30	8.74	13.43	29.27	46.17*
F_2 Generation					
0	6.38	9.32	14.01	29.32	47.75
10	6.02*	8.75	13.39	28.26	44.55
50	6.23	9.02	13.66	28.33	43.77*
500	6.22	8.99	13.28	28.06	42.99*

* $p<0.05$

For the F_1 litter, there was a statistically significant decrease in pup body weights at the low dose (10 ppm) at all time periods recorded. Since this effect was not dose-related, this reviewer does not consider it to be due to treatment. The statisically significant decrease seen at the high dose at day 21 in body weights is also not considered, by this reviewer as well as the study author, as treatment related since it too is not dose related. However, in the F_2 generation, the statistically significant decrease in pup body weights at day 21 in the mid and high dose are considered to be treatment-related by this reviewer, since there appears to be a dose-related effect on pup body weights at this time period and in this generation.

b. External observations of pups during lactation: Pups were observed daily during lactation. No treatment-related effects

were seen.

c. Sacrifice and necropsy of pups: Pups culled on postnatal day 4 were subjected to gross necopsy as were 40 randomly selected F_2 pups on day 21. No treatment-related findings were noted.

d. Other reproductive parameters: The following reproductive paramenters were studied: number of viable litters, litter size, stillbirths, sex ratio, surival indices, male and female fertility, male and female mating index, number of pregnant females, number of implantation sites, number of viable newborns and post-implantation loss. None of these parameters was affected by treatment (see Appendix 2, Tables 6.6.3., 6.6.4., 6.8.1., 6.14.3., 6.14.4., and 6.16.1. taken from the study report).

C. CONCLUSIONS:

Atrazine at dietary levels of 10, 50, and 500 ppm had no effect on the reproductive parameters studied; however, pup weights at postnatal day 21, second generation were statistically significantly lower than those of the control group at 50 and 500 ppm. The significance of these body weight effects could have been better addressed if two litters had been produced in each generation. In the absence of this information, the reduced pup weights at this time point are considered to be treatment-related.

Body weights, body weight gain and food consumption were statistically significantly decreased for parental animals, males and females, throughout the study at the HDT. These are considered to be treatment-related effects. In addition the statistically significant increase in relative testes weights could be treatment-related, since this effect was seen in both parental generations.

Parental NOEL = 50 ppm
Parental LEL = 500 ppm based upon decreased body weight, body weight gain, and food consumption for parental animals throughout the study. In addition, the increase in relative testes weights could be treatment-related, since this effect was seen in parental males of both generations.

Reproductive NOEL = 10 ppm
Reporductive LEL = 50 ppm based upon decreased body weight of pups on postnat day 21 in the second generation.

D. CORE CLASSIFICATION: Core-Minimum

Appendix 1

ATRAZINE 080803

Page_____ is not included in this copy.

Pages 182 through 188 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

V. **DISCUSSION:**

Atrazine exposure did not cause a dose-related increase in the incidence of neoplasms in the CD-1 strain of mice in this study. No dose-related effects are seen for macroscopic gross lesions or ocular changes in either sex during the 91-week atrazine feeding study.

The NOEL and the LEL are determined on the following bases. The LEL is set at 1500 ppm based upon decreases of 23.5% and 11.0% in mean body weight gain found at 91 weeks in male and female mice, respectively. Also, an increase in the incidence of cardiac thrombi is found in female mice in the 1500 ppm exposure group. None of the above effects are found at 300 ppm, thus the NOEL is set at 300 ppm.

This oncogenicity study shows that there are dose-related effects of atrazine in mice fed chow containing 1500 ppm or 3000 ppm atrazine. The dose-related effects are the production of cardiac thrombi, a decrease in the mean body weight gain at 12 and 91 weeks during the study, and decreases in erythrocyte count, hematocrit and hemoglobin concentration. An increase in the incidence of cardiac thrombi is found in female in the 1500 ppm and 3000 ppm exposure groups. In addition to amyloidosis, cardiac thrombi contributed to the deaths of the group of mice who did not survive to terminal sacrifice (this group of mice are termed as "early death" mice). This assertion is based on the observation that a statistically significant increased incidence of cardiac thrombi is found in "early death" mice whereas no statistically significant increase in incidence of cardiac thrombi is found in the group of mice who survived to terminal sacrifice in the same exposure group. These responses are the only dose-related effects observed in these mice in this study.

Female mice in the 3000 ppm exposure group recieved almost twice the dietary intake levels of atrazine when compared to male mice in the 3000 ppm exposure group. This observation may explain the 25% survival of female mice and 60% survival of male mice in the 3000 ppm exposure group.

At the highest exposure level, 3000 ppm, atrazine exposure in both sexes of mice caused:

1) a decrease in the mean body weight gain at 12 and 91 weeks,

2) a decrease in food consumption rates,

3) an increase in the incidence of cardiac thrombi,

4) a decrease in erythrocyte count, hemoglobin concentration and hematocrit, and

in female mice only:

1) an increase in mortality,

2) a decrease in mean brain and kidney weights, and

3) decreased percentages of neutrophils and lymphocytes.

Both the 1500 ppm and the 3000 ppm atrazine exposure levels are deemed sufficient doses to cause an adequate level of toxicity in male and female mice because:

1) the high percentage of mortality at 91 weeks (75%) in female mice in the 3000 ppm atrazine exposure group,

2) decreased mean body weight gain at 91 weeks in female mice (48.5%) and in male mice (24.4%) fed chow containing 3000 ppm atrazine,

3) a 23.5% decrease in mean body weight gain in male mice and an 11.0% decrease in female mice in the 1500 ppm atrazine exposure group at 91 weeks, and

4) at 12 weeks a 33.0% decrease in mean body weight gain in male mice and a corresponding 14.3% decrease in female mice in the 1500 ppm exposure group.

This study was well conducted and has been deemed sufficient quality to determine the oncogenic potential of atrazine. This study should be given the core classification of "guideline" because the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §83-2 have been satisfied.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D C 20460

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MEMORANDUM: Addendum To Rat Teratology Study #60-84

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Atrazine - Company Response to Toxicology Branch Reviews of the Rat and Rabbit Teratology Studies. Submitted March 25, 1988 by Ciba-Geigy Corporation.
Tox. Branch Project No.: 8-0744 Tox. Chem. No.: 63

TO: Robert Taylor
Product Manager 25
Registration Division (TS-767C)

FROM: Judith W. Hauswirth, Ph.D.
Section Head, Section VI
Toxicology Branch/HED (TS-769C)

THRU: Theodore M. Farber, Ph.D., Chief
Toxicology Branch/HED (TS-769C)

Action Requested: Determine whether the submitted data justify upgrading the Core classification of the rat and rabbit teratology studies on Atrazine from Core Supplementary to Core Minimum.

Discussion:

1. Rabbit Teratology Study (MRID 405663-01)

This study was classified as Core Supplementary pending submission of the purity of the technical product. The registrant has submitted information indicating that the purity of the technical product was approximately 96.3%.

2. Rat Teratology Study (MRID 405663-02)

This study was classified as Core Supplementary pending submission of the purity of the technical product and since a NOEL for runting was not demonstrated. The registrant has submitted information indicating that the purity of the technical product was approximately 96.7% and historical control data to address the absence of a NOEL for runting in the study. The historical control data along with the incidence of runting in the study are summarized in the following table.

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Incidence of Runting

	Dosage Group (mg/kg) 0	10	70	700	Hist. Control[1]
No. of fetuses	292	350	351	67	8712
No. of runts	1(0.3)[2]	5(1.4)	7(2.0)	65(97)	90(1.0)[3]
No. of litters	23	23	25	5	663
No. of litters with runts	1(4.3)	3(13)	4(16)	5(100)	65(9.8)[4]

1 Data from 25 studies were submitted. Data from one study, conducted in 1981, was not included by this reviewer. Only data from studies conducted from 1982 to 1986 are summarized. The study under consideration was dated 1984.
2 Numbers in parentheses are percentage incidence.
3 Range = 0 - 4.0%
4 Range = 0-26.3%

The incidence of both runts and litters with runts was within the historical control range for the low and mid dose groups; however, for both of these parameters and dosage groups the incidence was slightly higher than the mean historical control value. The concurrent control values, on the other hand were low when compared to the historical control mean values. This reviewer feels that the incidence of runts in the low and mid dose group of this study is not treatment related, based upon the submitted historical control data and the comparatively low concurrent control values seen in the Atrazine study. In addition, the increase over control values in both the number of runts and the number of litters with runts was not statistically significant at the low or mid dose by Fischer's Exact test at the 0.05 level (Statistics done by this reviewer).

At the mid dose, there were statistically significant increases by both fetal and litter incidence in skeletal variations indicating delayed ossification. These included: skull not completely ossified, presphenoid not ossified, teeth not ossified, metacarpals not ossified, metacarpals bipartite, and distal phalanx not ossified. The incidences of these effects in the control and low dose group were comparable.

Conclusions:

1. Rabbit Teratology Study

The core grade of this study can be raised to Core Minimum.

2. Rat Teratology Study

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The core grade of this study can be raised to Core Minimum.

Developmental NOEL = 10 mg/kg
Developmental LEL = 70 mg/kg based upon an increased incidence of skeletal variations indicating delayed ossification.

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STUDY REVIEW

Chemical: Atrazine
Test Material: Atrazine Technical
Study/Action Type: Teratology study

STUDY IDENTIFICATION:

"A Teratology Study of Atrazine Technical in Charles River Rats"

Testing Facility: The Safety Evaluation Facility, CIBA-GEIGY Corp., Summit, New Jersey
Project No.: 60-84
Report Date: 9-15-84
Study Director: Robert N. Infurna, Ph.D.
EPA Accession No.: 253479 MRID 00143008
Study Reviewed by: Geraldine S. Danford, B.A. 11/87.
Helene B. Morgan, B.A.

BACKGROUND

The Safety Evaluation Facility, CIBA-GEIGY Corp., Summit, New Jersey, conducted this teratology study of Atrazine Technical in Charles River rats during the fall of 1984.

CONCLUSIONS

It is concluded that the teratology study of atrazine technical in rats (CIBA-GEIGY Corporation, # 60-84) demonstrates the following:

Maternal No Observed Effect Level (NOEL): 10 mg/kg/day
Maternal Lowest Observed Effect Level (LOEL): 70 mg/kg/day

These values are based on a statistically significant decrease in bod. weight gain during the first half of the dosing period and a statistically significant reduction in food consumption for the first two days of agent administration in the 70 mg/kg/day group. The maternal mortality at the highest dose tested, 700 mg/kg/day, was 77.8%, making this group unsatisfactory for evaluation.

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Developmental toxicity NOEL: 5 mg/kg/day
Developmental toxicity LOEL: 75 mg/kg/day

These values are based on a statistically significant increase in the number of resorptions in the high dose group, significantly decreased fetal weights (male and female) in this group, and an increase in skeletal variations, especially delayed ossification of appendicular skeletal elements.

PROCEDURES

Test material:	Atrazine technical
Vehicle:	3% aqueous corn starch containing 0.5% Tween 80
Dosage levels:	0, 1, 5, or 75 mg/kg/day by gavage
Period of administration:	Days 7-19 of gestation
Species:	New Zealand White rabbits

The protocol used in this study was in compliance with those recommended in the Standard Evaluation Procedure (SEP), Teratology Studies (EPA-540/9-85-018, June 1985).

Graded doses of atrazine were administered by gavage to virgin female rabbits which had been artificially inseminated using semen collected from males of the same strain maintained at the research site. The oral route of dosing was chosen because potential human exposure is by this route.

Dosing occurred daily and was performed on days 7 through 19 of pregnancy with the day of artificial insemination being counted as day 0. This dosing period agrees with the recommendations of the SEP and covers the period of organogenesis in the rabbit.

Identification of individual rabbits, housing, food, water, environment, quarantine time, and group assignments were of standard experimental design. Dose level was based on the animals' body weight recorded on gestational days 7 and 14. Dosing was performed as indicated in Table 1.

Table 1: Dosing schedule

Group number	Number of females	Days of treatment	Dose (mg/kg/day)
1 (control)	19	7-19	0
2	19	7-19	1
3	19	7-19	5
4	19	7-19	75

The control group (vehicle control) received 5 ml/kg/day of 3% corn starch with Tween 20, which was a volume equivalent to that received by rabbits treated with atrazine.

According to the SEP, the highest dose of three different levels should induce overt maternal toxicity but not more than 10% maternal death. The highest dose (75 mg/kg/day) did induce overt maternal toxicity. A significant decrease in weight gain and food consumption were found during and after treatment. Significant increases were also found in vaginal bleeding and in little, none and/or soft stools. No deaths were found in this treatment group. The lowest dose is not supposed to induce evidence of toxicity. In the 1 mg/kg/day group there were three unexpected deaths. One female died after being dosed on day 17 of gestation. Another was found dead on day 19, apparently the result of a dosing accident. The third female was found dead on day 26 of gestation (possibly aborting). None of the deaths were dose related and thus are not considered significant. All other findings were similar to controls.

All does were examined daily for changes in appearance, behavior and food consumption. Individual body weights were recorded on days 0, 7, 14, 19, 21, 25, and 29. On day 29, all surviving females were sacrificed and examined for corpora lutea, uterine content, and gross morphological changes.

The fetuses were numbered in order of their positions in the uterus. Apparently viable fetuses were weighed and examined for gross abnormalities. Each fetus was examined viscerally using Staples' technique and its sex was determined. Following the visceral examination all fetuses were stained for skeletal examinations to determine malformations and/or variations.

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RESULTS

A. MATERNAL EVALUATION

Maternal Mortality

The only three deaths in the entire study occurred in the lowest dose (1 mg/kg/day) group. None of the findings at necropsy indicated any relationship to the dose level, but rather to the process of dosing.

Clinical Observations

Stool changes were observed in low and intermediate dose group animals but were not considered to have been compound-related because the incidences were similar to that in the controls. All does in the high dose group exhibited stool changes. These changes were statistically significant and considered signs of maternal toxicity. Another statistically significant sign of toxicity in the high dose group was blood either on the vulva or in the cage in 4/19 females. (Table 2)

Incidental findings included alopecia, lacrimation, scabs, nasal discharge, vasodilation and decreased motor control. All of these findings did not appear to be dose-related.

Pregnancy Rates

The pregnancy rates for the 0, 1, 5, and 75 mg/kg/day groups were, respectively, 84.2%, 89.5%, 84.2%, and 94.7%. The pregnancy rates given are within acceptable ranges for rabbits. Woo and Hoar reported an 83% pregnancy rate among those artificially inseminated out of 1142 pregnancies in NZW rabbits.[1] All does were examined for uterine contents following death, moribund sacrifice, or sacrifice on gestation day 29, showing the pregnancy rate is based on total number of females inseminated and not only on those surviving until sacrifice on gestation day 29. (Table 3)

Maternal Body Weight Data

Individual maternal body weights were recorded on days 0, 7, 14, 19, 21, 25, and 29 of presumed gestation. Prior to the dosing period (days 0-7), no differences in body weight gain were noted in any group. (Table 4)

[1]Woo, O.C. and R.M. Hoar, Reproductive performance and spontaneous malformations in control New Zealand White rabbits: a joint study by MARTA, Teratology 25(2): 82A, 1982.

TABLE 2.
Summary of Clinical Observations in Does

Observations	Dose (mg/kg) 0	1	5	25
Stool: little, none and/or soft	9/19	4/19	10/19	19/19**
Blood on vulva/in Cage	0/19	1/19	0/19	4/19*
Vasodilation of Ears	0/19	0/19	0/19	1/19
Decreased Motor Activity	0/19	0/19	0/19	1/19
Alopecia	5/19	3/19	2/19	9/19
Lacrimation	3/19	0/19	1/19	3/19
Scab	1/19	1/19	2/19	0/19
Nasal Discharge	1/19	0/19	0/19	0/19
Abortion	0/19	0/19	1/19	2/19
Death	0/19	3/19	0/19	0/19

*Different from the control group at $p \leq 0.05$.
**Different from the control group at $p \leq 0.01$.

TABLE 3.
Summary of Reproductive Parameters

	Treatments (mg/kg/day)			
	0	1	5	75
No Pregnant	16	17	16	18
% Pregnant	84.2	89.5	84.2	94.7
Mean No. Corpora Lutea/Litter	13.6	13.1	12.9	14.3
Mean No. Implantations/Litter	10.1	10.2	10.5	10.4
No. Litters Examined	16	14	15	15
Mean No. Embryonic Resorptions	.40	.30	1.0	1.6
Mean No. Fetal Resorptions	.90	1.1	.40	3.2
Mean Number of Resorptions	1.3	1.4	1.4	4.8**
Mean Number of Dead Fetuses	0.0	0.0	0.0	0.0
Pre-Implantation Loss (No.)	3.6	2.9	2.5	3.9
Pre-Implantation Loss (%)	26.1	21.6	18.4	26.5
Post-Implantation Loss (%)	12.0	11.4	13.0	32.6**
Mean Number of Live Fetuses	8.8	8.9	9.1	5.9*
Fetal Sex Ratio (% Males)	48.6	47.6	44.1	51.7

*Different from the Control at $p \leq 0.05$.
**Different from the Control at $p \leq 0.01$.

TABLE 4.
Summary of Maternal Body Weight (grams)

Days of Gestation	Treatments (mg/kg/day)			
	Control (0)	1	5	75
0	3952 ± 81 (16)[a]	3798 ± 86 (14)	3854 ± 56 (15)	3999 ± 91 (15)
7	4038 ± 85 (16)	3972 ± 88 (14)	4019 ± 52 (15)	4181 ± 94 (15)
14	4151 ± 91 (16)	4049 ± 93 (14)	4083 ± 47 (15)	3659 ± 88* (15)
19	4270 ± 100 (16)	4153 ± 104 (14)	4128 ± 43 (15)	3454 ± 84* (15)
21	4316 ± 104 (16)	4192 ± 105 (14)	4171 ± 44 (15)	3545 ± 96* (15)
25	4337 ± 95 (16)	4225 ± 102 (14)	4238 ± 49 (15)	3903 ± 81* (15)
29	4363 ± 86 (16)	4261 ± 98 (14)	4280 ± 52 (15)	4012 ± 82* (15)
29U[b]	3779 ± 100 (16)	3685 ± 95 (14)	3711 ± 43 (15)	3605 ± 88 (15)

[a] Numbers in parenthesis ( ) equal number of animals used in mean.
[b] Day 29U = (Term.) Body Weight less uterus, placentas and fetuses.
*Different from the Control at $p \leq 0.01$.

Statistically significant reductions in maternal body weights were observed in the high dose group for gestational days 14-29 (Table 5). Also, weight gains in the high dose group were significantly reduced at some intervals during and following treatment. During the treatment (days 7-14 and days 14-19), body weight losses rather than body weight gains were found. The total body weight gain for the high dose group for the entire gestational period (days 0-29U) was also significantly reduced.

In the intermediate group (5 mg/kg/day) the body weight gain was significantly reduced for gestational days 14-19. Other weight variations in this group are not considered significant.

Any changes in the weights of does in the low dose group were not significant, as the average body weight of this group was never less than 97% of the average body weight of does in the control group.

Maternal Food Consumption Data

Maternal toxicity, as shown by a statistically significant reduction in food consumption during treatment, was observed in the nigh dose (75 mg/kg/day) group. (Table 6) Following treatment, evidence of recovery was observed as food consumption increased. This increase was statistically significant for days 24-28.

Although slight reductions in food consumption were also found in the intermediate group (5 mg/kg/day), these reductions were statistically significant only for gestational days 17 and 19.

Food consumption was very slightly reduced in the low dose group both before and during the dosing period, the reduction being statistically significant only on day 13 of gestation. Because these reductions also occurred before the dosing period, they were not considered to have been compound-related.

Abortion

There were three confirmed abortions in the present study. One intermediate dose female on day 21 and two high dose females on days 20 and 25 were killed because they were aborting.

One of the females who died in the low dose group was thought to possibly be aborting on day 26.

TABLE 5.
Summary of Maternal Weight Gain (grams)

Days of Gestation	Treatments (mg/kg/day)			
	Control (0)	1	5	75
0-7	185 ± 19 (16)[a]	174 ± 2[a] (14)	166 ± 16 (15)	182 ± 20 (15)
7-14	113 ± 13 (16)	76 ± 13 (14)	63 ± 18 (15)	-522 ± 19** (15)
14-19	120 ± 15 (16)	105 ± 17 (14)	45 ± 23* (15)	-204 ± 26** (15)
19-21	46 ± 16 (16)	39 ± 10 (14)	43 ± 10 (15)	108 ± 27 (14)
21-25	21 ± 22 (16)	33 ± 26 (14)	67 ± 13 (15)	304 ± 37** (13)
25-29	26 ± 21 (16)	36 ± 11 (14)	42 ± 16 (15)	109 ± 25* (15)
0-29U[b]	-73 ± 52 (16)	-113 ± 64 (14)	-143 ± 42 (15)	-393 ± 28** (15)

[a]Numbers in parenthesis ( ) equal number of animals used in mean.
[b]Day 29U = Day (Term.) Body Weight less uterus, placentas and fetuses.
*Different from the Control at $p \leq 0.05$.
**Different from the Control at $p \leq 0.01$.

TABLE 6.
Summary of Maternal Food Consumption (grams)

Days of Gestation	Control (0)	Treatments (mg/kg/day) 1	3	75
7	183 ± 11 (13)[a]	164 ± 10 (12)	185 ± 8 (15)	76 ± 8** (15)
8	200 ± 11 (15)	180 ± 8 (11)	183 ± 7 (15)	98 ± 7** (15)
9	185 ± 10 (12)	172 ± 11 (12)	175 ± 6 (14)	20 ± 6** (13)
10	201 ± 12 (13)	179 ± 10 (13)	176 ± 5 (15)	12 ± 3** (14)
11	177 ± 12 (15)	177 ± 8 (13)	170 ± 6 (14)	8 ± 3** (15)
12	182 ± 9 (14)	170 ± 8 (13)	162 ± 6 (15)	2 ± 1** (14)
13	182 ± 8 (13)	155 ± 8* (10)	163 ± 6 (15)	1 ± 1** (15)
14	175 ± 10 (13)	156 ± 13 (10)	156 ± 7 (15)	2 ± 1** (15)
15	181 ± 13 (12)	149 ± 10 (11)	157 ± 10 (15)	2 ± 1** (12)
16	177 ± 17 (10)	159 ± 13 (11)	162 ± 15 (13)	3 ± 1** (15)
17	182 ± 10 (12)	157 ± 14 (11)	138 ± 15* (14)	6 ± 2** (15)
18	167 ± 11 (9)	158 ± 11 (12)	137 ± 16 (14)	8 ± 8** (15)
19	168 ± 9 (14)	136 ± 10 (11)	129 ± 12* (14)	18 ± 7** (14)
20	161 ± 10 (14)	148 ± 13 (12)	136 ± 11 (14)	80 ± 16** (12)
21	148 ± 10 (15)	128 ± 11 (11)	138 ± 10 (15)	116 ± 18 (12)
22	142 ± 13 (16)	116 ± 14 (12)	132 ± 10 (14)	162 ± 18 (13)
23	129 ± 11 (16)	110 ± 14 (13)	115 ± 9 (13)	162 ± 18 (11)
24	127 ± 9 (14)	100 ± 12 (13)	128 ± 8 (14)	174 ± 9* (13)
25	92 ± 11 (16)	95 ± 11 (13)	96 ± 6 (13)	176 ± 10* (13)
26	91 ± 10 (15)	93 ± 11 (12)	95 ± 7 (13)	177 ± 11* (12)
27	80 ± 9 (13)	94 ± 9 (12)	100 ± 6 (13)	162 ± 15* (11)
28	61 ± 6 (10)	91 ± 11 (12)	87 ± 6 (13)	151 ± 15* (10)

[a] Numbers in parentheses ( ) equal number of animals used in mean.
*Different from the Control at $p \leq 0.05$.
**Different from the Control at $p \leq 0.01$.

Reproduction Data at C-Section

The results of cesarean section, including ovarian, uterine, and litter data, are presented in Table 3.

The mean number of corpora lutea, uterine implantations, and the mean implantation efficiency were comparable for all groups examined. For the 0, 1, 5, and 75 mg/kg/day groups the mean number of resorptions were, respectively, 1.3 (12.0%), 1.4 (11.4%), 1.4 (13.0%), and 4.8 (42.6%). The 75 mg/kg/day values were statistically significantly higher ($p<0.01$) than the control values. Except for the number of resorptions in the high dose group, the results compare favorably with control data in NZW rabbits compiled by Woo and Hoar.[2]

The mean number of live fetuses per litter was 8.8 (control), 8.9 (1 mg/kg/day), 9.1 (5 mg/kg/day), and 5.9 (75 mg/kg/day). The high dose group had a reduced number of fetuses, which is statistically significant ($p<0.05$) when compared to the control group.

B. DEVELOPMENTAL TOXICITY EVALUATION

Fetal Data

The fetal data collected at cesarean section are summarized in Table 3.

As noted earlier, only in the high dose group was a significant change in the litter size observed. No dead fetuses were found in the control or any of the treated groups. Also, no variations in fetal sex ratio were detected.

Only in the 75 mg/kg/day group was a statistically significant reduction of mean fetal weight observed. The male fetal weight was 35.7 grams ($p<0.01$) and the female fetal weight was 35.8 grams ($p<0.01$). These values were compared to 46.1 grams (males) and 44.0 grams (females) for the control fetuses. These data are shown in Table 7.

[2]Woo and Hoar, op. cit.

Table 7: Summary of Fetal Weights (grams)

Parameter	Treatments (mg/kg/day) 0	1	5	75
Fetal Weight - Male	46.1	44.0	43.2	35.7*
Fetal Weight - Female	44.0	42.3	42.1	35.6*

*Different from the control group at P<0.01.

The reviewers investigated a possible increase in the number of runts (body weight less than 30 grams) in the treated groups. The findings are presented in the table in Attachment I. A definite increase was found in the high dose group. A slight increase was found in both the 1 mg/kg/day and 5 mg/kg/day fetuses; however, no increase over controls was found in the number of litters containing runts for either of these two groups. Seven of the 9 runts in the 5 mg/kg/day dose group were in very large litters containing 11 or 12 fetuses, which often results in low birth weight. The other 2 were in a litter consisting of 9 fetuses. Additional calculations gave the mean total litter weight (combined weights of all fetuses in a litter added for dose level divided by total number of litters) for controls to be 387.8 grams and that for the 5 mg/kg/day group to be 334.75 grams. Many teratologists advocate the litter approach over the fetal approach in handling statistical data for determining the effect of an agent.[3-5] Information from researchers working with New Zealand White rabbits indicated that the control values of 4 runts in 4 of 16 litters was unusually high and the problem might be with the rabbits supplied for the study. Without support of other signs of fetal toxicity at the 5 mg/kg/day dose level, the reviewers conclude that, under the conditions of this study, no biologically significant effect of atrazine on the fetus at the intermediate dose level is evident.

[3] Haseman, H., Choice of number of sampling units in teratology. Teratology 9: 257-258, 1974.

[4] Staples, R.E., and J.K. Haseman, Selection of appropriate experimental units in teratology, Teratology 9: 259-260, 1974.

[5] Weil, C.S., Selection of the valid number of sampling units and a consideration of their combination in toxicological studies involving reproduction, teratogenesis or carcinogenesis, Food and Cosmetics Toxicology 8: 177-182, 1970.

Malformation and Variations

Of the 489 fetuses produced in this study, two were externally malformed, one had a visceral abnormality, and one had a skeletal malformation. (Table 8) The external anomalies were omphalocele (control group) and ablepharia (high dose group). The only visceral malformation was the absence of a gallbladder in one intermediate dose fetus. The only skeletal malformation was in one control fetus which exhibited ectromelia. All of these were considered spontaneous and not dose related.

A slight increase in skeletal variation was found with increasing dose levels. The parameters most increased were those concerning delayed ossification. The high dose group had statistically significant increases in delayed ossification. These findings are indicative of fetal toxicity secondary to severe maternal toxicity, a conclusion consistent with reduced fetal body weights in the high dose group. (Table 9)

Discussion

These reviewers are in general agreement with the more important conclusions of this study. The means and standard deviations were found to be correct after spot checking.

Administration of technical atrazine to NZW rabbits from days 7-19 of gestation resulted in maternal toxicity during the treatment period at doses of 5 and 75 mg/kg/day. Does in the 75 mg/kg/day group did not recover from symptoms of this toxicity during the period after dosing. Signs of maternal toxicity in the intermediate dose group were decreased food consumption and decreased body weight. Signs of maternal toxicity in the high dose group included blood on vulva or in cage, decreased food consumption, abnormal stools, and decreased body weight and weight gain. In the lowest dose level (1 mg/kg/day) no dose related toxicity was observed. It can be concluded that a maternal NOEL in rabbits can be set at 1 mg/kg/day. The maternal LOEL in this study is 5 mg/kg/day.

The increased number of resorptions in the high dose group was statistically significant and was not observed in any of the other groups. No dead fetuses were observed in any of the groups. Fetal weights were normal in all groups except the high dose group. In this group the weights of both the male and female fetuses were significantly reduced. No compound-related malformations were observed. Skeletal variations, especially delayed ossification of appendicular skeletal elements, were found more frequently in the high dose group. Embryotoxicity and fetotoxicity in the high dose group was considered to have been secondary to maternal toxicity. These reviewers agree that the developmental toxicity (embryo/fetotoxicity) NOEL in rabbits is 5 mg/kg/day and conclude the LOEL is 75 mg/kg/day.

TABLE 8.
Summary of Fetal Malformations

Location	Parameter	Treatments (mg/kg/day) 0	1	5	75
	Total Number of Fetuses Examined	140	124	136	89
	Total Number of Litters Examined	16	14	15	15
External	Omphalocele	1	0	0	0
	Ablepharia	0	0	0	1
Visceral	Gallbladder-Absent	0	0	1	0
Skeletal	Ectromelia	1	0	0	0

TABLE 9.
Summary of Fetal Skeletal Variations (by Fetus)

Parameter	Treatments (mg/kg/day) 0	1	5	75
Number of Fetuses with Variations	94	80	99	75
Number of Fetuses with Variations Excluding Forepaw and Hindpaw	92	76	97	68

Attachment I.

Number of Runts (Body weight less then 30 grams)

Treatment mg/kg/day	0	1	5	75
Total litters	16	14	15	15
Male Runts	1	4	7	9
Female Runts	3	4	2	12
Total Runts	4	8	9	21
Number of litters with Runts	4	3	4	9

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D C 20460

006761

MEMORANDUM: Addendum to Rat Teratology Study #60-84

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Atrazine - Company Response to Toxicology Branch Reviews of the Rat and Rabbit Teratology Studies. Submitted March 25, 1988 by Ciba-Geigy Corporation.
Tox. Branch Project No.: 8-0744 Tox. Chem. No.: 63

TO: Robert Taylor
Product Manager #25
Registration Division (TS-767C)

FROM: Judith W. Hauswirth, Ph.D.
Section Head, Section VI
Toxicology Branch/HED (TS-769C)

THRU: Theodore M. Farber, Ph.D., Chief
Toxicology Branch/HED (TS-769C)

Action Requested: Determine whether the submitted data justify upgrading the Core classification of the rat and rabbit teratology studies on Atrazine from Core Supplementary to Core Minimum.

Discussion:

1. Rabbit Teratology Study (MRID 405663-01)

This study was classified as Core Supplementary pending submission of the purity of the technical product. The registrant has submitted information indicating that the purity of the technical product was approximately 96.3%.

2. Rat Teratology Study (MRID 405663-02)

This study was classified as Core Supplementary pending submission of the purity of the technical product and since a NOEL for runting was not demonstrated. The registrant has submitted information indicating that the purity of the technical product was approximately 96.7% and historical control data to address the absence of a NOEL for runting in the study. The historical control data along with the incidence of runting in the study are summarized in the following table.

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Incidence of Runting

	Dosage Group (mg/kg) 0	10	70	700	Hist. Control[1]
No. of fetuses	292	350	351	67	8712
No. of runts	1(0.3)[2]	5(1.4)	7(2.0)	65(97)	98(1.0)[3]
No. of litters	23	23	25	5	663
No. of litters with runts	1(4.3)	3(13)	4(16)	5(100)	65(9.8)[4]

1 Data from 25 studies were submitted. Data from one study, conducted in 1981, was not included by this reviewer. Only data from studies conducted from 1982 to 1986 are summarized. The study under consideration was dated 1984.
2 Numbers in parentheses are percentage incidence.
3 Range = 0 - 4.0%
4 Range = 0-26.3%

The incidence of both runts and litters with runts was within the historical control range for the low and mid dose groups; however, for both of these parameters and dosage groups the incidence was slightly higher than the mean historical control value. The concurrent control values, on the other hand were low when compared to the historical control mean values. This reviewer feels that the incidence of runts in the low and mid dose group of this study is not treatment related, based upon the submitted historical control data and the comparatively low concurrent control values seen in the Atrazine study. In addition, the increase over control values in both the number of runts and the number of litters with runts was not statistically significant at the low or mid dose by Fischer's Exact test at the 0.05 level (Statistics done by this reviewer).

At the mid dose, there were statistically significant increases by both fetal and litter incidence in skeletal variations indicating delayed ossification. These included: skull not completely ossified, presphenoid not ossified, teeth not ossified, metacarpals not ossified, metacarpals bipartite, and distal phalanx not ossified. The incidences of these effects in the control and low dose group were comparable.

Conclusions:

1. Rabbit Teratology Study

The core grade of this study can be raised to Core Minimum.

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2. Rat Teratology Study

The core grade of this study can be raised to Core Minimum. 006937

Developmental NOEL = 10 mg/kg
Developmental LEL = 70 mg/kg based upon an increased incidence of skeletal variations indicating delayed ossification.

83-8

00613:

STUDY REVIEW

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Chemical: Atrazine
Test Material: Atrazine Technical
Study/Action Type: Teratology study

STUDY IDENTIFICATION:

"A Teratology Study of Atrazine Technical in Charles River Rats"

Testing Facility: The Safety Evaluation Facility, CIBA-GEIGY Corp., Summit, New Jersey
Project No.: 60-84
Report Date: 9-15-84
Study Director: Robert N. Infurna, Ph.D.
EPA Accession No.: 254979 MRID 00143008
Study Reviewed by: Geraldine S. Danford, B.A. 4/11/87
Helene B. Morgan, B.A.

BACKGROUND

The Safety Evaluation Facility, CIBA-GEIGY Corp., Summit, New Jersey, conducted this teratology study of Atrazine Technical in Charles River rats during the fall of 1984.

CONCLUSIONS

It is concluded that the teratology study of atrazine technical in rats (CIBA-GEIGY Corporation, # 60-84) demonstrates the following:

Maternal No Observed Effect Level (NOEL): 10 mg/kg/day
Maternal Lowest Observed Effect Level (LOEL): 70 mg/kg/day

These values are based on a statistically significant decrease in bod. weight gain during the first half of the dosing period and a statistically significant reduction in food consumption for the first two days of agent administration in the 70 mg/kg/day group. The maternal mortality at the highest dose tested, 700 mg/kg day, was 77.8%, making this group unsatisfactory for evaluation.

(See supplemental memo)

Developmental Toxicity LOEL: 10 mg/kg/day

This value is based on a three-fold increase over controls in the number of litters containing runts. No historical control data was supplied in the report to show if the control group in this particular study experienced an unusually low rate of runting.

From the data furnished, under the conditions of this study, a Developmental Toxicity NOEL cannot be set.

PROCEDURES

Test material:	Atrazine Technical
Vehicle:	1% aqueous corn starch containing 0.5% Tween 80
Dosage levels:	0, 10, 70, 700 mg/kg/day by gavage
Period of administration:	Days 6-15 of gestation
Species:	Charles River CD rats

The protocol used in this study was in compliance with those recommended in the Standard Evaluation Procedure (SEP), Teratology Studies (EPA-540/9-85-018, June 1985).

Atrazine was administered once daily by gastric intubation to females mated to males of the same strain. The study was composed of four groups and the number of females per group was 27. This number was more than adequate as the minimum recommended number is 20. The oral route of dosing was chosen because potential human exposure is by this route.

Dosing occurred daily and was performed on days 6 through 15 of presumed gestation, the period of organogenesis in the rat. The day of mating was determined by the presence of sperm in the vaginal washing and was designated as day "0" of gestation.

Identification of individual rats, housing, food, water, environment, quarantine time, and group assignments were of standard experimental design. Dosing was performed as indicated in Table 1.

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Table 1: Dosing schedule

Group number	Number of females	Days of treatment	Dose (mg/kg/day)
1 (control)	27	6-15	0
2	27	6-15	10
3	27	6-15	70
4	27	6-15	700

The control group received 10 ml/kg/day of 3% corn starch containing 0.5% Tween 80 which was a volume equivalent to that received by treated rats. The volume of suspension of compound or vehicle to be administered to each animal was determined by the animal's body weight recorded on gestational days 6, 10, and 14.

According to the SEP, the highest dose of three different levels should induce overt maternal toxicity but not more than 10% maternal death. The highest dose in this study does not meet this requirement as 21 of the 27 females died during gestation.

All dams were observed daily for changes in appearance and behavior. Females were weighed on days 0, 6, 10, 14, 15, and 20 of gestation. Food consumption measurements were taken daily for gestational days 5 to 20. On day 20, the surviving dams were sacrificed and necropsied. A detailed examination and recording was made of the uterine contents. The females were examined for gross pathology and any maternal gross lesions were excised for microscopic evaluation.

The fetuses were numbered in order of their positions in the uterus from the ovarian end of the left horn to the ovarian end of the right horn. Apparently viable fetuses were weighed and the fetuses examined for gross abnormalities. Approximately 1/3 of the fetuses were fixed for visceral examination and 2/3 were prepared for skeletal examination.

RESULTS

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A. MATERNAL EVALUATION

Maternal Mortality

A high rate of maternal death (21/27) occurred in the 700 mg/kg/day group. This was 77.8% of the females in this group. No deaths were observed in the control, 10, or 70 mg/kg/day groups.

Clinical and Pathological Observations

No maternal toxicity was observed in the control group and the only toxic sign recorded in the 10 mg/kg/day group was rales in one dam. Although alopecia was statistically significant in the intermediate group it was not considered to be biologically significant since it is commonly observed in control animals at The Safety Evaluation Facility. Statistically significant symptoms observed in the high dose (700 mg/kg/day) group included: salivation in 13/27, oral/nasal discharge in 12/27, ptosis in 11/27, swollen abdomens in 8/27 and blood on the vulva in 7/27. Incidental findings in this group included alopecia and swollen hindleg. (Table 2)

At necropsy high-dose females were found to have a statistically elevated increase in enlarged stomachs (26/27), enlarged adrenals (12/27), and discolored lungs (3/27).

Pregnancy Rates

The pregnancy rates for the 0, 10, 70, and 700 mg/kg/day groups were, respectively, 88.9%, 85.2%, 92.6%, and 96.3%. The pregnancy rates given are within acceptable ranges for rats. Woo and Hoar reported a 70% pregnancy rate for pooled data on 1982 Charles River CD rats in control studies.[1] All females were examined for uterine contents following early death or sacrifice on gestation day 20, showing the pregnancy rate is based on total number of females mated and not only on those surviving until sacrifice on gestation day 20. (Table 3)

Maternal Body Weight Data

Individual maternal body weights were recorded on days 0, 6, 11, 14, 18, and day 20 of presumed gestation. (Table 4)

[1]Woo, D.C., and R.M. Hoar. Reproductive performance and spontaneous malformations in control Charles River CD rats: a joint study by MARTA, Teratology 19: 54A, 1979.

Prior to the dosing period, no differences in body weight gain were noted in any group. (Table 5) In the low dose group, a statistically significant increase in body weight gain was found during days 6-10 of gestation. In the intermediate group, body weight gains were significantly reduced during the first half of agent administration (days 6-10). Statistically significant reductions in bod. weights were observed in the high dose group on days 14, 18, and 20 of gestation. (Table 4) The body weight changes for the high dose group were also significantly reduced for gestation days 10-14, 14-18, 18-20, and 6-20. (Table 5)

Maternal liver weights in the high dose group were significantly reduced; however, this was not considered biologically significant because no significant dose-related differences were found for liver weight as a percentage of the day 20 body weight.

Maternal Food Consumption

Statistically significant reductions in food consumption were observed in the high dose (700 mg/kg/day) group during the dosing and post-dosing periods. This is considered a sign of severe maternal toxicity. The intermediate group (70 mg/kg/day) also had a significant reduction in food consumption for the first two days of compound administration (days 6 and 7). Statistically significant increases in food consumption were observed on gestation day 9 in the low dose group and on gestational day 17 (post-dosing) in the intermediate dose group. (Table 6)

Fetal Evaluation Data at C-Section

The results of cesarean section, including ovarian, uterine, and litter data, are presented in Table 7.

The mean number of corpora lutea, uterine implantations and resorptions, and mean implantation efficiency were comparable for the groups examined, except for some discrepancies found in data for the high dose group. These discrepancies seem to result from the high number of maternal deaths in this group and the various times during pregnancy at which the necropsy was performed. The reported results compare favorably with control data in Charles River CD rats compiled by Woo and Hoar.[2] The mean number of live fetuses per litter was 12.7 (control), 12.7 (10 mg/kg), 14.0 (70 mg/kg), and 12.4 (700 mg/kg).

[2]Woo and Hoar, op. cit.

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6. DEVELOPMENTAL TOXICITY EVALUATION

Fetal Data

The fetal data collected at cesarean section are summarized in Table 7.

As noted earlier, no significant change in the litter size was observed. No dead fetuses were found in the control, low or intermediate dose groups. In the high dose group only two fetuses in the same litter were found dead. No variations in fetal sex ratio were detected in any group.

Table 7: Summary of Fetal Weights (grams)

Parameter	Treatments (mg/kg day) 0	10	70	700
Fetal weight - Male	3.4	3.6	3.4	1.9*
Fetal weight - Female	3.2	3.2	3.2	1.8*

* Different from the control group at P 0.01.

[illegible] fetal weights in the low and intermediate groups are similar to the weights observed in the control group. (Table 7) In the control fetal weights were 3.4 grams (males) and 3.2 grams (females). These values are comparable with those compiled by Wise [illegible] 3.5 day fetuses (3.6 grams).[1] The high dose group [illegible] a statistically significant decrease in fetal weight [illegible] The weights were 1.9 grams (males) and 1.8 grams (females).

The researchers studied a possible increase in the number of litters [illegible] weight less than 2.5 grams found in the treated groups. The results of this study are presented in the table (attachment 1). In the 700 mg/kg/day group, 57 viable fetuses were included, and 4 out of these were runts. A trend toward [illegible] was found in 10 mg/kg/day and 70 mg/kg/day groups in both number of fetuses and the number of litters affected. These increases occurred in a dose-related manner.

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[1] Wise and Hoar, op. cit.

Malformations and Variations

External and visceral findings in all litters of this study are consistent with the data compiled by Woo and Hoar. In an examination of 28,142 fetuses they found only 49 (0.2%) exhibited external malformations. The incidences of visceral and skeletal malformations were 1.2% and 0.7%, respectively.[4] Banerjee and Durico also agreed that a low rate of malformations was observed in the Charles River CD rat and reported that no pronounced visceral anomalies were observed except for hydroureter (1.76%).[5]

Occurrence of malformations and variations are recorded in Table 5 and Table 6. Skeletal examinations were not conducted in the high dose group because fetal size and weight were severely reduced. Fetotoxicity, attributable to maternal toxicity, was observed in the intermediate group as exhibited by increased skeletal variations, considered developmental delays. While results of individual endpoints of ossification delay showed statistically significant increases in this group, these data were not significant when total number of fetuses with variations were compared on a fetal and litter basis. Four types of skeletal malformations were observed. Polydactyly and rib agenesis occurred in separate fetuses of the low dose group. Supernumerary thirteenth ribs occurred in six control, one low dose and two intermediate dose fetuses. Centrum/vertebrae agenesis occurred in three control fetuses. These malformations were not dose-related and are not considered compound related. Visceral variations in the treated groups were not increased significantly over those in the control groups. Thus there was no indication in this study that atrazine was teratogenic in the rat.

Conclusion

The administration of atrazine technical to Charles River CD rats from days 6-15 of gestation resulted in maternal toxicity during and after the treatment period at the 700 mg/kg/day dose level. Signs of toxicity in the high dose level group included death in 21 of 27 dams, reduced food consumption, reduced weight gain, salivation, ataxia, swollen abdomen, oral/nasal discharge, and bloody vulva. Maternal toxicity was also found at the 200 mg/kg/day dose level. Toxicity signs in this group included reduced food consumption, reduced body weight, and reduced weight gain. No maternal toxicity was observed in the 10 mg/kg/day or control groups. It can be concluded that a maternal NOEL in rats can be set at 10 mg/kg/day. The maternal LOEL in this study is 200 mg/kg/day.

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[4] Woo and Hoar, op. cit.

[5] Banerjee, B.N. and R.S Durico, Incidence of teratological anomalies in control Charles River C-D strain rats, Toxicology 1: 151-154, 1973.

The number of resorptions was not dose related in any of the groups. Only two fetuses were found dead and both of these were found in the high dose group; however, this was not statistically significant. The mean number of live fetuses and the sex ratios were unaffected by increasing dosage in the 0, 10, 75, and 750 mg/kg/day groups. Fetal size and body weight were severely reduced in the high dose group. This fetal toxicity was considered to occur as a result of severe maternal toxicity. The body weights of the other treatment groups were similar to the weights of the control group; however, a trend toward runting was observed in all three treated groups, occuring in a dose-related fashion. The increase in total number of runts is not statistically significant, but the increase in the percent of litters containing runts.* In this study, the 10 mg/kg/day group had 3 litters (12% of the litters containing runts, while the control group had 1 litter (4.2% of the litters containing runts. No skeletal examination was performed on the high dose fetuses due to their small size. However, a statistically significant increase in skeletal variations was observed in the intermediate group. This was considered the result of developmental delays. This type of developmental delay is usually not permanent and therefore not considered a malformation. No dose-related malformations were observed in any of the treatment groups. These reviewers conclude that, under the conditions of this study, the developmental toxicity NOEL of atrazine in rats is 10 mg/kg/day, based on an increase in runting. A developmental NOEL cannot be determined from this study from the data provided.

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* [illegible] of the valid number of sampling units and [illegible] information in toxicity studies [illegible] statistical [illegible] teratogenesis. [illegible] [illegible] [illegible].

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006718

Reviewed by: Judith W. Hauswirth, Ph.D., Section Head Judith W Hauswirth 4/28/88
Section VI, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 2-Generation Reproduction Study (83-4) TOX. CHEM. NO.: 63

MRID NO.: 404313-03

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NO.: 852063

SPONSOR: Ciba-Geigy Corp., Agricultural Division, Greensboro, NC 27419

TESTING FACILITY: Research Department, Pharmaceuticals Divison, Ciba-Geigy Corporation, Summit, NJ 07901

AUTHORS: J Mainiero. M Youreneff, MLA Giknis and ET Yau

REPORT ISSUED: November 17, 1987

CONCLUSION: Parental NOEL = 50 ppm
Parental LEL = 500 ppm based upon decreased body weights, body weight gain, and food consumption in both parental males and females throughout the study. In addition, the increase in relative testes weights seen in parental males could be treatment-related since it was seen in both generations.

Reproductive NOEL = 10 ppm
Reproductive LEL = 50 ppm based upon decreased body weights of pups of the second generation on postnatal day 21.

CORE CLASSIFICATION: Core - Minimum

A. MATERIALS:

1. Test compound: Atrazine, Technical. Batch No. 41802. White powder. Purity not specified but stated to be on record at Ciba-Geigy Corporation, Greensboro, NC.

2. Test animals: Species: rat; Strain: Charles River (CRCD, VAF/PLUS) from Charles River Laboratory, Ltd., Kingston, NY; Age: 37 days; Weight: males, 177-219g and females, 140-176g; animals were quarantined for one week.

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B. STUDY DESIGN:

1. Animal assignment: According to the report, "One hundred twenty male and one hundred twenty female rats from the acclimation colony were randomly assigned a TEROS® temporary animal identification number and at the same time were randomly distributed into 4 treatment groups..." Permanent numbers were assigned when the animals were found acceptable for the study.

2. Experimental design: Male rats were placed on the control and test diets at 47 days of age and females at 48 days of age. They were maintained on these diets for a period of 10 weeks prior to mating. Males and females were housed together in a 1:1 ratio for mating. They were allowed a three week period for mating and were separated once evidence of mating was seen. One litter was produced in each generation. After weaning of the last litter of the first generation, thirty males and thirty females were selected for the second parental generation. The remaining male parental animals were sacrificed on days 113-114 of the study. The remaining female parental animals were sacrificed on days 133-134 of the study.

   Animals selected for the second parental generation were exposed to test diets for 12 weeks prior to mating. Mating was conducted in the same manner as for the first generation. Parental males were sacrificed on day 138 of the study and parental females on days 138, 139 and 152 after weaning of their litters.

3. Test diet: Atrazine was mixed with Purina #5002 Certified Rodent Chow. The concentrations used were 0, 10, 50 and 500 ppm. Diets containing 10 to 3000 ppm atrazine were found to be stable at room temperature for at least 40 days. Periodic homogeneity analyses were performed and atrazine concentrations were found to be 93-105% of the expected values.

4. Statistics: Statistical methods can be found in Appendix 1 (Section 2.13 of the report).

5. A signed quality assurance statement was included with the study report.

C. METHODS, RESULTS, AND DISCUSSION:

1. Parental animals:

   a. Observations: Animals were observed once daily for signs of toxicity and twice daily for mortality. No treatment-related clinical signs were seen in either parental generation. Alopecia and sore(s)/scab(s) were commonly seen in all groups including the controls.

      At the levels tested, atrazine had no effect on mortality in either parental generation.

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b. Body weights: Body weights were determined weekly and at termination for males. For females, body weights were recorded weekly during the premating phase, on days 0, 7, 14 and 20 of gestation and on days 0, 4, 7, 14, and 21 of lactation. Selected body weight data can be found summarized in the following table for both parental generations.

Selected Parental Body Weight Data

Dose (mg/kg)	Mean Body Weights (g) Day 0	21	49	70	Terminal
			Males, F_0		
0	198.4	339.0	448.8	500.9	566.3
10	198.1	338.5	449.8	508.9	577.8
50	197.6	337.4	447.8	501.2	567.8
500	198.0	309.3*	396.0*	440.7*	484.8*
			Males, F_1		
0	167.8	337.6	478.4	541.1	642.3
10	160.8	329.8	471.6	528.5	626.2
50	160.6	325.2	462.6	529.6	627.4
500	146.7*	294.7*	408.9*	459.5*	540.1*
			Females, F_0		
0	158.0	220.8	261.2	281.7	
10	154.8	215.2	258.4	280.0	
50	155.2	209.8	254.5	269.9	
500	154.2	197.5*	231.6*	243.5*	
			Females, F_1		
0	141.7	212.0	262.8	287.8	
10	138.7	216.4	272.1	296.1	
50	140.1	212.4	264.5	290.4	
500	127.9*	193.7*	232.7*	251.8*	

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Body Weights (Cont'd.)

	Females, F_0 (Gestation)		Females, F_0 (Lactation)		
	0	20	0	14	21
0	289.5	407.0	330.5	354.2	341.3
10	285.3	415.9	323.5	348.2	333.9
50	291.5	410.0	320.8	344.9	331.3
500	250.6*	376.6*	288.3*	319.1*	314.7*
	Females, F_1 (Gestation)		Females, F_1 (Lactation)		
0	302.0	408.4	329.8	347.5	333.7
10	298.5	413.4	334.7	344.5	335.3
50	305.3	418.1	341.4	346.7	333.3
500	260.8*	370.3*	297.6*	316.9*	315.2*

* $p<0.05$

Body weights were statistically significantly lower for both males and females fed the diet containing 500 ppm atrazine (HDT) throughout the study. Body weight gains were also statistically significantly depressed at the HDT. At the mid dose (50 ppm) sporadic statistically significant decreases in body weight gain were noted. These changes are not considered to be related to treatment since they were occasional and very sporadic.

c. Food consumption: Food consumption was determined weekly for males and females during the premating period and on days 0, 7, 14, and 20 of gestation for the females.

Food consumption was statistically significantly reduced for males and females during the premating period for both parental generations and for F_1 females on days 0-7 of gestation.

d. Sacrifice and pathology: All parental animals were subjected to gross pathological examination. The testes and ovaries were weighed. The following tissues were collected for microscopic examination:

vagina	cervix	ovaries
testes	epididymides	seminal vesicles
prostate	pituitary	coagualtion gland
gross lesions		

Tissues from the control and high dose group were examined microscopically as well as <u>all</u> gross lesions.

1) Organ weights: There were no treatment-related effects on ovarian weights. Relative but not absolute testes weights were statistically significantly increased at the HDT

in parental males of both generations. The study authors attributed this change to decreased body weight gain at this dosage level.

2) Gross necropsy: No treatment-related effects were seen in either generation.

3) Histopathology: No treatment-related effects were seen in either generation.

2. Reproductive effects:

a. Pup weights: Mean pup weights per litter were recorded on lactation days 0, 4, 7, 14, and 21. Selected overall mean pup weights for each dosage group and each generationn are shown in the following table.

Mean Pup Weights (g)

F_1 Generation	Day				
Dosage Group (ppm)	0	4 (pre-culling)	7	14	21
0	6.42	9.11	14.43	31.00	49.87
10	5.99*	8.10*	12.95*	28.31*	45.09*
50	6.17	8.56	13.54	29.87	47.23
500	6.30	8.74	13.43	29.27	46.17*
F_2 Generation					
0	6.38	9.32	14.01	29.32	47.75
10	6.02*	8.75	13.39	28.26	44.55
50	6.23	9.02	13.66	28.33	43.77*
500	6.22	8.99	13.28	28.06	42.99*

* $p<0.05$

For the F_1 litter, there was a statistically significant decrease in pup body weights at the low dose (10 ppm) at all time periods recorded. Since this effect was not dose-related, this reviewer does not consider it to be due to treatment. The statisically significant decrease seen at the high dose at day 21 in body weights is also not considered, by this reviewer as well as the study author, as treatment related since it too is not dose related. However, in the F_2 generation, the statistically significant decrease in pup body weights at day 21 in the mid and high dose are considered to be treatment-related by this reviewer, since there appears to be a dose-related effect on pup body weights at this time period and in this generation.

b. External observations of pups during lactation: Pups were observed daily during lactation. No treatment-related effects

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were seen.

c. Sacrifice and necropsy of pups: Pups culled on postnatal day 4 were subjected to gross necopsy as were 40 randomly selected F_2 pups on day 21. No treatment-related findings were noted.

d. Other reproductive parameters: The following reproductive paramenters were studied: number of viable litters, litter size, stillbirths, sex ratio, surival indices, male and female fertility, male and female mating index, number of pregnant females, number of implantation sites, number of viable newborns and post-implantation loss. None of these parameters was affected by treatment (see Appendix 2, Tables 6.6.3., 6.6.4., 6.8.1., 6.14.3., 6.14.4., and 6.16.1. taken from the study report).

C. CONCLUSIONS:

Atrazine at dietary levels of 10, 50, and 500 ppm had no effect on the reproductive parameters studied; however, pup weights at postnatal day 21, second generation were statistically significantly lower than those of the control group at 50 and 500 ppm. The significance of these body weight effects could have been better addressed if two litters had been produced in each generation. In the absence of this information, the reduced pup weights at this time point are considered to be treatment-related.

Body weights, body weight gain and food consumption were statistically significantly decreased for parental animals, males and females, throughout the study at the HDT. These are considered to be treatment-related effects. In addition the statistically significant increase in relative testes weights could be treatment-related, since this effect was seen in both parental generations.

Parental NOEL = 50 ppm
Parental LEL = 500 ppm based upon decreased body weight, body weight gain, and food consumption for parental animals throughout the study. In addition, the increase in relative testes weights could be treatment-related, since this effect was seen in parental males of both generations.

Reproductive NOEL = 10 ppm
Reporductive LEL = 50 ppm based upon decreased body weight of pups on postnatal day 21 in the second generation.

D. CORE CLASSIFICATION: Core-Minimum

Appendix 1

ATRAZINE 080803

Page_____ is not included in this copy.

Pages 227 through 234 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

CONFIDENTIAL BUSINESS INFORMATION
DOE No. Contract
NATIONAL SECURITY INFORMATION (EO 12065,

006937 84-2

EPA: 68-02-4225
DYNAMAC No. 2304-3
February 27, 1987

005833

TB Doc 5833

DATA EVALUATION RECORD

~~Salmonella~~ E.coli

ATRAZINE

Mutagenicity--In vitro Microbial Assays
(Bacillus subtilis Rec Assay and Escherichia coli and
Salmonella typhimurium Reverse Mutation Assays)

STUDY IDENTIFICATION: Sutou, S., Kimura, Y., Yamamoto, K., and Ichihara, A. In vitro microbial assays for mutagenicity testing of atrazine. (Unpublished study No. NRI-79-2884 prepared by Nomura Research Institute, Japan, for CIBA-GEIGY (Japan) Ltd., Japan; dated August 1979.) Accession No. 284052. MRID ~~00161931~~ 00161931

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 2-27-87

1. CHEMICAL: Atrazine; 2-chloro-4-ethylamino-6-isopropylamino-S-triazine.

2. TEST MATERIAL: Atrazine, from lot No. G 30027, was described as a white powder with a purity of 98.8%.

3. STUDY/ACTION TYPE: Mutagenicity--In vitro microbial assays (Bacillus subtilis rec assay and Escherichia coli and Salmonella typhimurium reverse mutation assays).

4. STUDY IDENTIFICATION: Sutou, S., Kimura, Y., Yamamoto, K., and Ichihara, A. In vitro microbial assays for mutagenicity testing of atrazine. (Unpublished study No. NRI-79-2884 prepared by Nomura Research Institute, Japan, for CIBA-GEIGY (Japan) Ltd., Japan; dated August 1979.) Accession No. 284052.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 2-27-87

Brenda Worthy, M.T.
Independent Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 2-27-87

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 2-27-87

Henry Spencer, Ph.D.
EPA Reviewer

Signature: Henry Spencer
Date: 3/9/87

Altin Kocialski, Ph.D.
EPA Section Head

Signature: A. Kocialski
Date: 3/20/87

7. CONCLUSIONS:

Atrazine was investigated in a series of three microbial assays. The conclusions for each assay are presented below:

1. Bacillus subtilis Rec Assay

Six nonactivated doses of atrazine were assayed for the ability to induce DNA damage/repair in B. subtilis H17 and M45. Under the conditions of the assay, the test material did not cause preferential inhibition of repair-deficient M45 when compared to the parental and repair-competent H17 strain. However, the assay was not performed using S9 activation; therefore, the study is incomplete.

We conclude that the assay provides acceptable evidence of a non-genotoxic effect for the nonactivated test material; however, it is unacceptable as an overall screening procedure for genotoxic effects.

2. Escherichia coli Reverse Mutation Assay

Under the conditions of this assay, 50, 100, 500, 1000, 2000, and 5000 µg/plate nonactivated and S9-activated atrazine did not induce reversion to tryptophan prototrophy. The test material was assayed both to the limits of solubility (≥1000 µg/plate +/-S9) and to a level causing cytotoxicity (5000 µg/plate +/-S9).

We conclude that this assay is acceptable.

3. Salmonella typhimurium Reverse Mutation Assay

Seven doses of atrazine ranging from 50 to 10,000 µg/plate, both in the presence and absence of S9 activation, did not cause an appreciable increase in histidine revertants of S. typhimurium TA1535, TA100, TA1538, TA98, or TA1537. However, the results were compromised for the following reasons:

1. The S9-activated spontaneous reversion frequencies for TA1535, TA1538, TA98, and TA1537 were abnormally high and well beyond acceptable ranges.[1]

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[1] de Serres, F. J. and Shelby, M. D. Recommendations for data production and analysis using the Salmonella/microsome mutagenicity assay. Environ. Mutagenesis 1(1979):87-92.

2. The concentration of essential nutrients available to TA100 was approximately tenfold less than is recommended to ensure that several rounds of DNA replication occurred.[2] Thus, the spontaneous reversion frequency and sensitivity of the strains to detect the mutagenic effects, if any, of an unknown test material are equivocal.

3. Although evidence of cytotoxicity was noted, particularly at precipitating doses, the data were difficult to interpret because of conflicting results for individual strains and among the tester strains.

We conclude, therefore, that the S. typhimurium assay is unacceptable.

8. RECOMMENDATIONS:

The following recommendations are made to upgrade additional studies:

a. The B. subtilis assay should be conducted in the presence and absence of S9 activation to make the study fully acceptable Hence, the entire assay should be repeated.

b. The S. typhimurium assay should be repeated in accordance with recommended procedures.[3]

c. Since the combined study results indicated that the limit of solubility was achieved at 1000 µg/plate, both with and without S9 activation, the evaluation of higher test doses is not necessary.

d. A QA/GLP statement is required for future studies.

Items 9 and 10--see footnote [4].

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods. (See Appendix A for details.)

1. Test Material: Atrazine, lot No. G 30027, was described as a white powder with 98.8% purity; the powder was stable at 20°C for at least 12 months. The test material was dissolved in dimethylsulfoxide (DMSO).

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[2] Maron, D. M. and Ames, B. N. Revised methods for the Salmonella mutagenicity test. Mutat. Res. 113(1983):173-215.

[3] Ibid.

[4] Only items appropriate to this DER have been included.

2. Bacterial Strains: B. subtilis H17 and M45, E. coli WP2 Hcr⁻, and S. typhimurium TA1535, TA100, TA1537, TA1538, and TA98 were used in these studies. Neither the source nor storage conditions for the strains were reported. Overnight nutrient broth cultures of each strain were prepared for the assays.

3. S9 Activation: The S9 fraction used for metabolic activation was derived from the livers of Wistar male rats induced with "Tetra" (500 mg/kg of polychlorobiphenyl). The S9 mix was prepared according to Nagao et al.[5] on the day of use.

4. Microbial Assays

   a. B. subtilis Rec Assay: The DNA-repair assay was conducted with six nonactivated doses of the test material. Overnight broth cultures of the repair-competent H17 and repair-deficient M45 strains were diluted 1:2 and streaked radially over the surface of nutrient broth agar plates prepared to contain a central, 1 cm, concaved well. Fifty-microliter volumes of the six selected test doses, the solvent control (DMSO), the negative controls (7.5 N NaOH, 2 N HCl, and 100 µg/well Kanamycin, KM), or the positive control (10 µg/well 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, AF-2) were added to the central well on the appropriate plates. It was noted that tabular presentation of the data indicated that 100-µL volumes were applied to the wells.

   The plates were incubated overnight at 37°C; the length of the inhibitory zones were measured and the difference in growth between M45 and H17 for respective treatment groups was compared.

   b. E. coli Reverse Mutation Assay: Six concentrations of the test material were assayed both in the presence and the absence of S9 activation in the E. coli reverse mutation assay. One-tenth milliliter of an overnight broth culture of E. coli, grown to a density of 3.3 x 10^9 cells/mL, was inoculated into tubes containing 2.5 mL of molten top agar (0.6% NaCl, 0.8% Difco agar, and 1 µg/mL tryptophan). Bacterial inoculation was followed by the addition in 0.1-mL volumes, of the selected doses of the test material, solvent control

[5] Nagao, M., Yahagi, T., Seino, Y., Sugimura, T., and Ito, N. Mutat. Res. 42(1977): 335.

(DMSO), or positive controls (2 µg/plate N-methyl-N'-nitro-N-nitrosoguanidine, MNNG, -S9, or 20 µg/plate 2-aminoanthracene, 2AA, +S9).

For the S9-activated assay, an unspecified amount of S9 mix was incorporated into the reaction mixtures. The contents of each tube were mixed and poured over Vogel-Bonner medium E (VBE); duplicate plates were prepared for all treatments. Plates were incubated at 37°C for 3 days. At the end of incubation, the number of revertant colonies was counted and averages were calculated.

c. S. typhimurium Reverse Mutation Assay: The S. typhimurium reverse mutation assay was conducted with six S9-activated and nonactivated doses of the test material. Overnight broth cultures of the appropriate tester strain in 0.1-mL volumes were added to tubes containing 2.5-mL volumes of molten top agar supplemented with histidine and biotin. For unexplained reasons, the final concentration of histidine and biotin in top agar prepared for strain TA100 was tenfold less (0.005 mM histidine/biotin) than the recommended concentration.[6] It was noted, however, that the remaining strains were inoculated into top agar prepared to contain the required concentration of essential nutrients (0.05 mM histidine/biotin).

Following strain inoculation, 0.1 mL of the appropriate test material dilution, solvent (DMSO), or positive controls were added. The amount of S9 mix added to the top agar tubes for the S9-activated assay was not reported. The following mutagens were used as the positive controls:

Strain	Mutagen	Concentration (µg/plate)	S9 Activation
TA1535	β-propiolactone (β-PL)	100	-
TA100, TA98	AF-2	0.1	-
TA1538	2-Nitrofluorene (2-NF)	50	-
TA1537	9-Aminoacridine (9-AA)	200	-
TA1535, TA100, and TA98	2AA	20	+
TA1538, TA1537	2AA	10	+

[6] Maron, M. and Ames, B. N. Mutat. Res. 113(1983):173-215.

The contents of each tube were mixed and overlaid onto VBE medium; plates were incubated at 37°C for 2 days. Revertant colonies were counted and the average number of his^+ colonies were determined from the counts of duplicate plates for each treatment group.

4. Evaluation Criteria

a. B. subtilis Rec Assay: No specific criteria to evaluate assay validity or a positive response were reported. The positive control was judged genotoxic based on a 10-mm difference in inhibition by M45 when compared to H17.

b. E. coli Reverse Mutation Assay: No specific criteria to evaluate assay validity or a positive response were reported. The positive controls were judged mutagenic based on the marked increase in the number of tryptophan revertants when compared to the negative control.

c. S. typhimurium Reverse Mutation Assay: Although no specific criteria for a positive response were reported, it was deduced from the authors' comments that a compound was considered mutagenic if a ≥2-fold increase in his^+ colonies was accompanied by a dose-related effect. Based upon the increase in revertant colonies when compared to the solvent control, the authors considered the positive controls to be mutagenic.

B. Protocol: A protocol was not presented.

12. REPORTED RESULTS:

a. B. subtilis Rec Assay: The six doses selected for the nonactivated Rec assay were 100, 500, 1000, 2000, 5000, and 10,000 µg/well. The authors reported compound precipitation at doses ≥500 µg/well. Neither the test material doses nor the DMSO control were cytotoxic for either strain. Approximately equal zones of inhibition, indicative of cytotoxicity, were recorded for the negative controls (7.5 N NaOH, 2 N HCl, or 100 µg/well KM). By contrast, preferential inhibition of repair-deficient M45 when compared to repair-competent H17 was observed following exposure to the positive control, 10 µg/well AF-2. Representative results of the Rec assay are shown in Table 1.

b. E. coli Reverse Mutation Assay: The six nonactivated and S9-activated doses evaluated in the E. coli reverse mutation assay were 50, 100, 500, 1000, 2000, and 5000 µg/plate. In both the presence and absence of S9 activation, compound precipitation was reported for doses ≥1000 µg/plate.

TABLE 1. Representative Results of the Bacillus subtilis Rec Assay with Atrazine

Substance	Dose/Well	Zone of inhibition (mm) of B. subtilis strains[a] H17 (Rec+)	M45 (Rec-)	Difference (mm)[b]
Solvent Control				
Dimethylsulfoxide	100 µL	0	0	0
Negative Control				
NaOH (7.5N)	100 µL	21.5	22.5	+ 1
HCl (2N)	100 µL	20.0	20.5	+ 0.5
Kanamycin	100 µg	8.5	9.0	+ 0.5
Positive Control				
2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide	10 µg	10.0	22.3	+ 12.3[c]
Test Material				
Atrazine	10,000 µg[d]	0	0	0

[a] Average length of zones from duplicate plates for all test conditions but the highest assayed dose (results from single plates presented for 10,000 µg/well); average zone lengths were calculated by our reviewers.

[b] Difference = (Average zone for M45) - (average zone for H17); calculated by our reviewers.

[c] Judged positive by the authors.

[d] Highest assayed dose; compound precipitation reported for doses ≥500 µg/mL; results for lower doses (100, 500, 1000, and 5000 µg/well) were comparable to the solvent control.

Cytotoxicity, as indicated by a greater than 40% reduction in tryptophan revertants, was observed at the highest dose both with and without S9 activation. No evidence of cytotoxicity was noted below this concentration and no appreciable increase in revertant colonies of E. coli WP2 Hcr$^-$ resulted from exposure to the five nonactivated and S9-activated test doses. Both MNNG (2 µg/plate, -S9) and 2AA (20 µg/plate, + S9) were mutagenic. Representative results are presented in Table 2.

c. S. typhimurium Reverse Mutation Assay: Presented in Table 2 are representative results of the S. typhimurium reverse mutation assay. For this study, seven concentrations ranging from 50 to 10,000 µg/plate were assayed with and without S9 activation. In agreement with the findings of the E. coli reverse mutation assay, doses ≥1000 µg/plate (+/-S9) precipitated. In the absence of S9 activation, the two highest doses (5000 and 10,000 µg/plate) were cytotoxic for all strains except TA1537. Strain TA100 appeared to be more sensitive to the cytotoxic effects of the nonactivated test material (35% reduction in his$^+$ colonies at 1000 µg/plate) than the remaining strains.

In the presence of S9 activation, cytotoxicity was generally confined to the highest dose for the majority of strains; however, a 50% reduction in his$^+$ colonies of strain TA1537 was evident at 5000 µg/plate. In contrast to the nonactivated results, which indicated that TA100 was the most sensitive to cytotoxic action, S9-activated atrazine appeared more cytotoxic to strain TA15[illegible]; however, the dose response was reversed (i.e., more cytotoxic at 100 µg/plate; less cytotoxic with increased test material doses).

No appreciable increase in his$^+$ colonies of any strain accompanied exposure to the graded dose of the test material, either with or without S9 activation. (Data were omitted for strain TA100 at the 5000-µg/plate level, +S9). All strains responded to the appropriate direct-acting or metabolically activated mutagen.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded, "Atrazine was not mutagenic in any of the test systems used. Atrazine is concluded to be a nonmutagenic under the present experimental conditions."

B. A quality assurance statement was not provided.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that within the confines of these studies, atrazine was not mutagenic; however, individual studies were compromised either by the absence of key elements or by specific technical problems.

TABLE 2. Representative Results of the Escherichia coli and Salmonella typhimurium Reverse Mutation Assays with Atrazine

Substance	S9 Activation	Dose (µg/plate)	Revertants per Plate of Bacterial Tester Strain[a]					
			E. coli WP2 Hcr⁻	S. typhimurium TA1535	TA1537	TA1538	TA98	TA100
Control								
Dimethylsulfoxide	-	0	19	26	14	33	37	1[illegible]
	+	0	18	60	35	93	105	2[illegible]
Positive Controls								
N-Methyl-N'-nitro-N-nitrosoguanidine	-	2	1,620	-	-	-	-	-
β-Propiolactone	-	100	-	790	-	-	-	-
9-Aminoacridine	-	200	-	-	2,667	-	-	-
2-Nitrofluorene	-	50	-	-	-	2,089	-	-
2-(2-Furyl)-3-(5-nitro-2-furyl)-acrylamide	-	0.1	-	-	-	-	520	1,8[illegible]
2-Aminoanthracene	+	20	295	577	-	-	-	-
	+	10	-	-	217	1,065	1,588	1,24[illegible]
Material								
Urine	-	100[b]	19	23	14	33	33	10[illegible]
	+		22	27	47	101	108	15[illegible]
	-	5000[c]	11	14	7	24	23	76
	+		6	44	19	88	93	-
	-	10,000[d]	-	15	10	23	18	52
	+		-	43	11	50	56	10[illegible]

[a] Average count of duplicate plates.

[b] Highest dose showing evidence of cytotoxicity (strain TA1535 only, +S9).

[c] Highest dose assayed with E. coli; values for lower doses (50, 500, 1000, and 2000 µg/plate) were comparable to the solvent control.

[d] Highest dose assayed with S. typhimurium; values for remaining doses (50, 500, 1000, and 2000 µg/plate) were comparable to the solvent control.

[e] Compound precipitation was reported for doses ≥1000 µg/plate.

B. subtilis Rec Assay: Atrazine did not cause cellular DNA damage in the two B. subtilis strains; however, the lack of exogeneous metabolic activation renders the study incomplete and precludes an overall assessment of the DNA-damaging potential of the test material in the Rec assay.

E. coli Reverse Mutation Assay: Atrazine was assayed both to the limits of solubility and to a cytotoxic dose with no mutagenic effect in an acceptable study.

S. typhimurium Reverse Mutation Assay: The following technical problems were uncovered in reviewing the S. typhimurium reverse mutation assay:

1. The spontaneous revertant frequencies for TA1535, TA1537, TA1538, and TA98 in the presence of S9 activation were abnormally high and exceeded acceptable ranges.

2. Although the control values for TA100 were acceptable, the 90% reduction in available histidine and biotin (see Section II.A.5c) probably lowered the actual rate of spontaneous reversion; hence, the numbers do not reflect the true background counts for TA100. Of added concern is the probable reduction in strain sensitivity caused by the decreased histidine. The recommended concentration of histidine in the top agar was established to guarantee that both auxotrophs and prototrophs undergo several divisions. Since DNA replication is necessary in many cases for mutagenesis, the lack of sufficient amino acid may have obscured the results with TA100 and led to a false negative conclusion.

3. The cytotoxicity findings were difficult to interpret because of the conflicting results reported for certain strains and the differences among strains (i.e., not cytotoxic for TA1537 -S9; but cytotoxic for +S9; marked cytotoxicity for TA100 -S9 but reduced cytotoxicity for +S9; moderately cytotoxic for TA1535 -S9 but increasingly cytotoxicity with a negative dose response for +S9). It is possible that compound precipitation at the three highest doses interfered with accurate mutant counts and data from concentrations >1000 ug/plate should probably be excluded from the evaluation.

We conclude, however, that the problems associated with high background frequencies for TA1535, TA1537, TA1538, and TA98 and the reduction in essential nutrients provided to strain TA100 render the findings unsupportable evidence of a nonmutagenic response.

Item 15--see footnote 4.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 57-59.

07-2

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY TB Occ.
WASHINGTON, D.C. 20460 006368

SEP 21 1987 006937

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

Subject: Atrazine - Submission of Additional Mutagenicity Data in Response to the Registration Standard. Submitted by Ciba-Geigy. June 19, 1987. Accession No.: 264052; MRID 402466-01.
Tox. Project No.: -0902 Tox. Chem. No.: 63

To: Robert Taylor
Product Manager #25
Registration Division (TS-767C)

From: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 9/14/87.
Section Head, Section VI
and
Irving Mauer, Ph.D. JWH for Irving Mauer 9/14/87
Section VI
Toxicology Branch/HED (TS-769C)

Thru: Theodore M. Farber, Ph.D., Chief
Toxicology Branch/HED (TS-769C)
9/15/87

Action Requested: Review a recently conducted Ames Salmonella assay conducted on atrazine and consider arguments presented by Ciba-Geigy to support the adequacy of two previously reviewed mutagenicity assays (UDS in rat hepatocytes and dominant lethal in mice) that were judged by Toxicology Branch to be unacceptable.

Conclusions/Recommendations:

X 1. Ames Salmonella Assay: Acceptable and negative up to 5000 ug/plate (the "limit dose" at which slight toxicity was observed). MRID 402466-01

X 2. UDS in Rat Hepatocytes: Ciba-Geigy's responses are acceptable, as provided data in the supplement to the original report (dated October 86). This study is now considered to be acceptable. MRID 402466-02

3. Dominant Lethal Assay in Mice: Ciba-Geigy's responses are not acceptable for concluding that atrazine was adequately tested in this assay. An acceptable assay in the category of chromosomal aberrations is still a data gap for atrazine.

A detailed review follows.

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Detailed Reveiw 006937

Study Title: Atrazine: Salmonella/Mammalian - Microsome Mutagencity Test

Author: E. Deparade

Report Dated: December 5, 1986

Conducting Laboratory: Ciba-Geigy Limited, Basle, Switzerland

Study Number: 861172

Test Material: G 30027 Technical; purity, 98.2%; Batch No., Lot 210200

Vehicle: Dimethylsulfoxide

Procedure: The assay was conducted according to the methods of Ames, et al. (ref. 1-3). The strains tested were TA 98, TA 100, TA 1535 and TA 1537 both with and without metabolic activation. The metabolic activation system was derived from the S9 fraction of liver from Tif:RAIf(SPF) rats treated with Aroclor 1254. Positive controls consisted of the following:

Without metabolic activation

TA 98	daunorubicin-HCl
TA 100	4-nitroquinoline-N-oxide
TA 1535	sodium azide
TA 1537	9(5)-aminoacridine hydrocnloride monohydrate

With metabolic activation

TA 98	2-aminoanthracene
TA 100	2-aminoanthracene
TA 1535	cyclophosphamide
TA 1537	2-aminoanthracene

A signed quality assurance statement was included with the report. Historical control data for each strain were also included.

Preliminary Toxicity Screen: The toxicity screen was conducted with TA 100 in the absence of metabolic activation. Concentrations ranged from 0.08 to 5000 ug/0.1 ml. One/tenth of an ml was added to each plate.

Concentrations of Test Material: 20, 78, 313, 1250 and 5000 ug/0.1 ml both with and without metabolic activation.

Criteria for a Positive Response: (The following is taken directly from the report).

The test substance is considered to be positive in this test system if one or both of the following conditions are met:

- a reproducible doubling of the mean number of revertants per plate above that of the negative control at any concentration level for one or more of the following strains: TA 98, TA 1535 and TA 1537,

- a reproducible increase of the mean number of revertants per plate for any concentration above that of the negative control by a factor of 1.5 for strain TA 100.

Generally a concentration-related effect should be demonstrable.

Results: In the preliminary toxicity screen, no toxicity was seen up to 5000 ug/plate (the limiting dose) and this was, therefore, chosen as the highest dose to be tested.

No increase in the incidence of histidine-prototrophic mutants in comparison with the negative control was seen with any of concentrations of G 30027 tested. Minimal toxicity was seen at 5000 ug/plate for all strains tested both with and without metabolic activation.

Conclusions: This is an acceptable assay, indicating that G 30027 is negative when tested up to 5000 ug/plate in the Salmonella/ mammalian - microsome mutagenicity test.

Study Title: Atrazine: Autoradiographic DNA Repair Test on Rat Hepatocytes

This study was completed on May 16, 1984 and submitted to the Agency on July 28, 1986. It was reviewed by Toxicology Branch and their comments were transmitted to Ciba-Geigy by letter on April 23, 1987. The study was considered to be unacceptable for the following reasons:

a. "The combined 24-hour hepatocyte attachment period and 5-hour test compound exposure time caused a marked reduction in assay sensitivity as indicated by the less than adequate response of the positive control;
b. Cytoplasmic background grain counting was not performed;
c. Slides were not coded".

Ciba-Geigy has submitted arguments/additional data to address the above points. These include:

a. "Both time periods used appear to be appropriate in that the positive control assays provided a clear, consistent positive response. These responses, as well as a number of historical control values can be compared in the supplement to the report [The supplement was included in the submission.];
b. Cytoplasmic background grain counts are provided in the supplement to the report [The supplement was included in the submission.];
c. The slides were not coded; however, they were counted electronically thereb eliminating bias".

After reviewing the submitted data, Toxicology Branch concludes that the assay

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can now be considered acceptable for showing that G 30027 does not induce UDS in rat hepatocytes.

Study Title: Atrazine: Dominant Lethal Test

This study was originally submitted to the Agency on July 28, 1986. It was reviewed by Toxicology Branch and found to be unacceptable. Our comments were transmitted to Ciba-Geigy by letter on April 23, 1987. With this submission, Ciba-Geigy has responded to our comments indicating that they believe that the assay should be considered acceptable.

Toxicology Branch Comment:

Under the conditions of the study, 444 or 1332 mg/kg G30 027, administered by gavage to male mice, did not elicit a dominant lethal effect. However, G30 027 did not induce a toxic or a cytotoxic effect. Therefore, we are unable to assess whether the test material reached the target organ (gonads). Ciba-Geigy's Response:

There is nothing in the TSCA test guideline requirement (40CFR part 798.5450) citing the need for determining whether the test substance reached the gonads. (A systemically administered compound would be expected to reach all organ systems.)

Toxicology Branch Comment:

Although performed and reported in 1981, this study was not submitted until 1986, at which time the current Test Guidelines (September 27, 1985) had been in effect for at least a year. According to these current Guidelines ["at least]....3 dose levels are employed, the highest should produce signs of toxicity, either clinical (in treated males), or reduced fertility (in untreated pregnant females)." Neither procedure was followed in this test, and no evidence given that any portion of the orally administered single dose reached all organ systems in effective amounts (to cause either target toxicity or mutagenic events).

Ciba-Geigy Response:

As stated in a footnote on page 5 of the report, the approximate oral LD_{50} of atrazine in mice is 3992 mg/kg. The present study conducted at levels of 1332 and 444 mg/kg used doses that were 33 and 11 percent of the LD_{50} dose, respectively. The dose of 1332 mg/kg would be expected to elicit systemic toxicity based on findings of sedation, dyspnea, piloerection, and hunched appearance observed in mice treated with a dose of 1670 mg/kg (see refereence 1). From these data, it would appear that the maximum tolerated dose would be exceeded at a level of 1332 mg/kg.

Toxicology Branch Comment:

The high dose of 1332 mg/kg is indeed quite high, but produced no effects in any of the treated males. The selection of doses was stated to have been based on Reference 1 (attached to the back of the report): "Acute

Oral LD_{50} of Tech. Atrazine in the Mouse", an undated study conducted with a different batch of test chemical with an unstated purity and, hence, not appropriate for dose selection.

006937

Ciba-Geigy Response:

In the September 24, 1986 Federal Register (Vol. 51, No. 158, p. 34009), it is stated that "the Agency will place greater weight on tests conducted in germ cells than in somatic cells, on tests performed in vivo rather than in vitro, in eukaryotes rather than prokaryotes, and in mammalian species rather than in submammalian species." The attached test report fulfills or exceeds those criteria.

Toxicology Branch Comment:

The "criteria" described here refer to an overall assessment of mutagenic potential for man, and not to a scientific evaluation of any particular mutagenicity assay (where current Test Guidelines govern).

Further, this dominant lethal study is deficient in not sampling the entire spermatogenic cycle of the mouse, acknowledged by seasoned investigators as more closely approximating 8 weeks, not the six weeks of matings employed in this study. That 8 weeks of mating was standard practice even in the laboratory is re-enforced in the additional data submitted as a supplement (starting on p. 52 of the present version of the study report), on a positive control group performed in the same year as this atrazine study, detailing the results of 8 weeks of mating.

Conclusion:

This study remains unacceptable for the reasons outlined in the Toxicology Branch comments above.

References:

1. Ames, BN, Lee, FD and Durston, WE (1973), An Improved Bacterial Test System for the Detection and Classification of Mutagens and Carcinogens. Proc. Natl. Acad. Sci. USA 70:782-786.

2. Ames, BN, Durston, WE, Yamasaki, E and Lee, FD (1973), Carcinogens are Mutagens: A Simple Test System Combining Liver Homogenates for Activation and Bacteria for Detection. Proc. Natl. Acad. Sci. USA 70, 2281-2285.

3. Ames, BN, McCann, J and Yamasaki, E (1975), Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian Microsome Mutagenicity Test. Mutation Res. 31:347-364.

II. DETAILED REVIEW

006937

A. Test Material - G30-027 Technical (ATRAZINE)

Description: (Not stated)
Batch (Lot): 210200
Purity (%): 98.2
Solvent/carrier/diluent: Suspended in 0.5% carboxymethylcellulose (CMC) for oral administration.

B. Test Organism - Rodent

Species: Mouse
Strain: Tif:MAGF, SPF--NMRI-derived
Age: (Not stated)
Weights--Males: 30 to 34 g
Females: 21 to 30 g
Source: Ciba-Geigy Tierfarm, Sisseln (Switzerland)

C. Study Design (Protocol) - A formal protocol was not included in this report, but a list of authoritative publications was appended.

Both a Quality Assurance statement as well as a testament of compliance to FIFRA GLPs were included in the Final Report.

D. Procedure/Methods of Analysis - Following a preliminary toxicity test (up to acute doses of 5000 mg/kg) to select the highest dose, the main assay was performed in two parts. In the first part, 24 male and 24 female mice were intubated once with 2250 mg/kg test material, and groups of 8/sex sacrificed 16, 24, or 48 hours postdosing. In the second portion, groups of animals (8/sex) were administered atrazine at acute doses of 562.5, 1175, and 2250 mg/kg and sacrificed 24 hours later. Both a negative control group (CMC vehicle) and positive control, cyclophosphamide (CP, 64 mg/kg) were included in each part of the study.

At sacrifice, bone marrow was flushed from both femurs of each animal, spread on glass microscopic slides, and air-dried. The next day, the slides were stained with May-Grunwald, counter-stained with diluted Geimsa, rinsed in water, cleared in xylene, and mounted under coverslips.

Coded slides from 5 animals/sex/treatment were scored for micronuclei in 1000 polychromatic erythrocytes (m-PCE) per animal and the ratio of PCE to normochromatic

erythrocytes (PCE/NCE) determined. A test substance was considered positive if there was a statistically significant increase (by chi-square) in micronucleated PCEs at any dose or sampling time.

III. RESULTS

In preliminary toxicity testing, doses of 3750 mg/kg atrazine and above were lethal, but mice survived 2250 mg/kg.

In the first part of the main study, four females due to be sacrificed at 16 hours and three scheduled for the 24-hour kill died shortly after receiving 2250 mg/kg. Hence, only four females were available for scoring m-PCEs at the 5-hour sampling; an extra male was included for this time group. In the second part of the mutagenicity assay, one mid-dose female (1125 mg/kg) died before her scheduled sacrifice (24 hours).

No statistically significant increases over vehicle controls (i.e., $p > 0.05$) in m-PCEs were recorded in any test group at any sampling time in either portion of the study (summary tabulations from the Final Report are attached to this (DER), and no apparent effects on PCE/NCE ratio (a measure of cytotoxicity in bone marrow erythropoiesis) were found.

By contrast, CP-treated animals manifested 25- to 75-fold increases ($p < 0.05$) over their respective controls, again without compromising erythropoiesis.

The author concluded that atrazine was negative for inducing micronuclei in mice intubated up to acute doses causing death.

IV. TB EVALUATION

This two-part study appeared to have been conducted with adequate procedures and appropriate controls, rendering valid the interpretation of the results. Although no significant cytotoxicity (bone marrow erythropoiesis) was demonstrated, the test material was administered up to a dose causing death (2250 mg/kg), and the positive controls responded as expected with significant increases in micronuclei. Hence, the negative result with atrazine is assessed as valid, and the study ACCEPTABLE.

Attachments

ATRAZINE 080803

Page_____ is not included in this copy.

Pages 253 through 254 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D C 20460

006937

SEP 21 1987

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

Subject: Atrazine - Submission of Additional Mutagenicity Data in Response to the Registration Standard. Submitted by Ciba-Geigy. June 19, 1987. Accession No.: 264052: MRID 402466-01.
Tox. Project No.: 7-0902 Tox. Chem. No.: 63

To: Robert Taylor
Product Manager #25
Registration Division (TS-767C)

From: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 9/14/87
Section Head, Section VI
and
Irving Mauer, Ph.D. Irving Mauer 9/14/87
Section VI
Toxicology Branch/HED (TS-769C)

Thru: Theodore M. Farber, Ph.D., Chief
Toxicology Branch/HED (TS-769C)

Action Requested: Review a recently conducted Ames Salmonella assay conducted on atrazine and consider arguments presented by Ciba-Geigy to support the adequacy of two previously reviewed mutagenicity assays (UDS in rat hepatocytes and dominant lethal in mice) that were judged by Toxicology Branch to be unacceptable.

Conclusions/Recommendations:

1. ~~Ames Salmonella Assay: Acceptable and negative up to 5000 ug/plate (the "limit dose" at which slight toxicity was observed).~~ MRID 402466-01

2. UDS Assay in Rat Hepatocytes: Ciba-Geigy's responses are acceptable, as provided by data in the supplement to the original report (dated October 14, 1986). This study is now considered to be acceptable. MRID 402466-02

3. ~~Dominant Lethal Assay in Mice: Ciba-Geigy's responses are not acceptable for concluding that atrazine was adequately tested in this assay. An acceptable assay in the category of chromosomal aberrations is still a data gap for atrazine.~~

~~A detailed review follows.~~

Detailed Review

006937

Study Title: Bacterial Salmonella Mammalian - Microsome Mutagenicity Test

Author: E. DeGrace

Report Dated: December 5, 1984

Conducting Laboratory: Ciba-Geigy Limited, Basle, Switzerland

Study Number: 86[illegible]

Test Material: G 30027 Technical; purity, 98.2%; Batch No., Lot 210200

Vehicle: Dimethylsulfoxide

Procedure: The assay was conducted according to the methods of Ames, et al. (ref. 1-3). The strains tested were TA 98, TA 100, TA 1535 and TA 1537 both with and without metabolic activation. The metabolic activation system was derived from the S9 fraction of liver from Tif:RAIf(SPF) rats treated with Aroclor 1254. Positive controls consisted of the following:

Without metabolic activation

TA 98	daunomycin-HCl
TA 100	4-nitroquinoline-N-oxide
TA 1535	sodium azide
TA 1537	9(5)-aminoacridine hydrochloride monohydrate

With metabolic activation

TA 98	2-aminoanthracene
TA 100	2-aminoanthracene
TA 1535	cyclophosphamide
TA 1537	2-aminoanthracene

A signed quality assurance statement was included with the report. Historical control data for each strain were also included.

Preliminary Toxicity Screen: The toxicity screen was conducted with TA 100 in the absence of metabolic activation. Concentrations ranged from 0.08 to 5000 ug/0.1 ml. One/tenth of an ml was added to each plate.

Concentrations of Test Material: 20, 78, 313, 1250 and 5000 ug/0.1 ml both with and without metabolic activation.

Criteria for a Positive Response: (The following is taken directly from the report).

The test substance is considered to be positive in this test system if one or both of the following conditions are met:

- a reproducible doubling of the mean number of revertants per plate above that of the negative control at any concentration level for one or more of the following strains: TA 98, TA 1535 and TA 1537,

- a reproducible increase of the mean number of revertants per plate for any concentration above that of the negative control by a factor of 1.5 for strain TA 100.

Generally a concentration-related effect should be demonstrable.

Results: In the preliminary toxicity screen, no toxicity was seen up to 5000 ug plate (the limiting dose) and this was, therefore, chosen as the highest dose to be tested.

No increase in the incidence of histidine-prototrophic mutants in comparison with the negative control was seen with any of concentrations of G 30027 tested. Minimal toxicity was seen at 5000 ug/plate for all strains tested both with and without metabolic activation.

Conclusions: This is an acceptable assay, indicating that G 30027 is negative when tested up to 5000 ug/plate in the Salmonella/ mammalian - microsome mutagenicity test.

Study Title: Atrazine: Autoradiographic DNA Repair Test on Rat Hepatocytes

This study was completed on May 16, 1984 and submitted to the Agency on July 28, 1986. It was reviewed by Toxicology Branch and their comments were transmitted to Ciba-Geigy by letter on April 23, 1987. The study was considered to be unacceptable for the following reasons:

a. "The combined 24-hour hepatocyte attachment period and 5-hour test compound exposure time caused a marked reduction in assay sensitivity as indicated by the less than adequate response of the positive control;
b. Cytoplasmic background grain counting was not performed;
c. Slides were not coded".

Ciba-Geigy has submitted arguments/additional data to address the above points. These include:

a. "Both time periods used appear to be appropriate in that the positive control assays provided a clear, consistent positive response. These responses, as well as a number of historical control values can be compared in the supplement to the report [The supplement was included in the submission.];
b. Cytoplasmic background grain counts are provided in the supplement to the report [The supplement was included in the submission.];
c. The slides were not coded; however, they were counted electronically there eliminating bias".

After reviewing the submitted data, Toxicology Branch concludes that the assay

[illegible] considered acceptable for showing that G 30027 does not induce [illegible] in mice.

Study Title: Atrazine: Dominant Lethal Test

This study was originally submitted to the Agency on July 28, 1986. It was reviewed by Toxicology Branch and found to be unacceptable. Our comments were transmitted to Ciba-Geigy by letter on April 23, 1987. With this submission, Ciba-Geigy has responded to our comments indicating that they believe that the assay should be considered acceptable.

Toxicology Branch Comment:

Under the conditions of the study, 444 or 1332 mg/kg G30 027, administered by gavage to male mice, did not elicit a dominant lethal effect. However, G30 027 did not induce a toxic or a cytotoxic effect. Therefore, we are unable to assess whether the test material reached the target organ (gonads). Ciba-Geigy's Response:

There is nothing in the TSCA test guideline requirement (40CFR part 798.5450) citing the need for determining whether the test substance reached the gonads. (A systemically administered compound would be expected to reach all organ systems.)

Toxicology Branch Comment:

Although performed and reported in 1981, this study was not submitted until 1986, at which time the current Test Guidelines (September 27, 1985) had been in effect for at least a year. According to these current Guidelines ("at least'....3 dose levels are employed, the highest should produce signs of toxicity, either clinical (in treated males), or reduced fertility (in untreated pregnant females)." Neither procedure was followed in this test, and no evidence given that any portion of the orally administered single dose reached all organ systems in effective amounts to cause either target toxicity or mutagenic events).

Ciba-Geigy Response:

As stated in a footnote on page 5 of the report, the approximate oral LD_{50} of atrazine in mice is 3992 mg/kg. The present study conducted at levels of 1332 and 444 mg/kg used doses that were 33 and 11 percent of the LD_{50} dose, respectively. The dose of 1332 mg/kg would be expected to elicit systemic toxicity based on findings of sedation, dyspnea, piloerection, and hunched appearance observed in mice treated with a dose of 1871 mg/kg (see refereence 1). From these data, it would appear that the maximum tolerated dose would be exceeded at a level of 1332 mg/kg.

Toxicology Branch Comment:

The high dose of 1332 mg/kg is indeed quite high, but produced no effects in any of the treated males. The selection of doses was stated to have been based on Reference 1 (attached to the back of the report): "Acute

[illegible] Test, Mutation in the "Mouse", an unpublished study conducted with a [illegible] of test chemical with an unstated purity and, hence, not appropriate for dose selection.

Citizenship Response:

In the September 24, 1986 Federal Register (Vol. 51, No. 186, p. 34009), it is stated that "the Agency will place greater weight on tests conducted in germ cells than in somatic cells, on tests performed in vivo rather than in vitro, in eukaryotes rather than prokaryotes, and in mammalian species rather than in submammalian species." The attached test report fulfills or exceeds those criteria.

Toxicology Branch Comments:

The "criteria" described here refer to an overall assessment of mutagenic potential for man, and not to a scientific evaluation of any particular mutagenicity assay (where current Test Guidelines govern).

Further, this dominant lethal study is deficient in not sampling the entire spermatogenic cycle of the mouse, acknowledged by seasoned investigators as more closely approximating 8 weeks, not the six weeks of matings employed in this study. That 8 weeks of mating was standard practice even in the laboratory is re-enforced in the additional data submitted as a supplement (starting on p. 52 of the present version of the study report), on a positive control group performed in the same year as this atrazine study, detailing the results of 8 weeks of mating.

Conclusion:

This study remains unacceptable for the reasons outlined in the Toxicology Branch comments above.

References:

1. Ames, BN, Lee, FD and Durston, WE (1973), An Improved Bacterial Test System for the Detection and Classification of Mutagens and Carcinogens. Proc. Natl. Acad. Sci. USA 70:782-786.

2. Ames, BN, Durston, WE, Yamasaki, E and Lee, FD (1973), Carcinogens are Mutagens: A Simple Test System Combining Liver Homogenates for Activation and Bacteria for Detection. Proc. Natl. Acad. Sci. USA 70, 2281-2285.

3. Ames, BN, McCann, J and Yamasaki, E (1975), Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian Microsome Mutagenicity Test. Mutation Res. 31:347-364.

WC. 5833

EPA: 68-02-4225
DYNAMAC No. 230A-6
February 27, 1987

006937

DATA EVALUATION RECORD

ATRAZINE

Mutagenicity--Unscheduled DNA Repair in Primary Rat Hepatocytes

STUDY IDENTIFICATION: Puri, E. and Muller, D. Autoradiographic DNA repair test on rat hepatocytes with G 30 027, technical. (Unpublished study No. 831171 prepared and submitted by CIBA-GEIGY Ltd., Basle, Switzerland; dated May 16, 1984.) Accession No. 284052. MRID 40246602

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 2-27-87

006937

1. CHEMICAL: Atrazine; G 30 027.

2. TEST MATERIAL: G 30 027 technical was from batch No. P 210200 and had a purity of 98.2%; no further details were provided.

3. STUDY/ACTION TYPE: Mutagenicity--Unscheduled DNA repair in primary rat hepatocytes.

4. STUDY IDENTIFICATION: Puri, E. and Muller, D. Autoradiographic DNA repair test on rat hepatocytes with G 30 027, technical. (Unpublished study No. 831171 prepared and submitted by CIBA-GEIGY Ltd., Basle, Switzerland; dated May 16, 1984.) Accession No. 284052.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 2-27-87

Brenda Worthy, M.T.
Independent Reviewer
Dynamac Corporation

Signature: Brenda Worthy for
Date: 2-27-87

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 2-27-87

Henry Spencer, Ph.D.
EPA Reviewer

Signature: Henry Spencer
Date: 3/9/87

Albin Kocialski, Ph.D.
EPA Section Head

Signature: A. Kocialski
Date: 3/12/87

In support of this recommendation, Barfknecht et al.[5] recently demonstrated that an 18-hour exposure period was superior to shorter intervals for detecting the DNA repair elicited in response to chemicals such as 4-nitroquinoline-1-oxide, mitomycin C, and dimethylnitrosamine, which are well known for their DNA-damaging activity.

It is noteworthy that the concentration of DMN (100 mM) selected by the authors to demonstrate a positive 13.5-fold increase in UDS over the control was approximately 1000 times higher than the level used by Barfknecht et al. ($1x10^{-4}$ M) to affect a response 4 hours posttreatment, comparable to that reported in this study. The latter showed that after 18 hours, $1x10^{-4}$ M DMN induced a 40-fold increase. From a comparison of the study authors' results and those of Barfknecht et al. using DMN, we assess that the combination of a 24-hour attachment period and a 5-hour exposure period caused a marked reduction in assay sensitivity. Of equal concern is the extreme difference in the positive control concentration and the highest assayed dose of G 30 027; the DMN dose was 50 times higher than the highest concentration of the test material (150 µg/mL).

3. Cytoplasmic background grain counts were not counted. These data are essential because silver grains are not homogeneously distributed over the slides; therefore, the only way to correct for random grain distribution is to count and subtract the grains of adjacent cytoplasmic areas from the nuclear counts.

4. The slides were not coded.

We conclude, therefore, that the study should be repeated in accordance with recommended procedures for the primary rat hepatocyte UDS assay.

Item 15--See footnote 2.

16. CBI APPENDIX: ~~Appendix A, Material and Methods,~~ CBI pp. 5-7.

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[5] Barfknecht, T. R., Naismith, R. W. and Kornburst, D. J. Variations on the standard protocol design of the hepatocyte DNA repair assay. (Manuscript submitted to the J. Appl. Toxicol.)

TABLE 1. Representative Results of the Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay with G 30 027

Substance	Dose	No. of Cells Scored	Average Silver Grains/ Nucleus
Negative Control Untreated cells	-	150	1.61
Solvent Control Ethanol	-	150	1.61
Positive Control Dimethylnitrosamine	100 µM	150	21.8[a]
Test Material G 30 027	150 µg/mL[b]	150	1.42

[a]Positive by authors' criterion (>2-fold increase in the number of silver grains/nucleus over the control).

[b]Highest dose assayed; compound precipitation was reported for this dose in the preliminary cytotoxicity assay. Values for lower doses (1.2, 6, and 30 µg/mL) were slightly lower than the controls.

12. REPORTED RESULTS:

A. Preliminary Cytotoxicity Assay: The seven doses of the test material examined in the preliminary cytotoxicity assay ranged from 3.125 to 150 µg/mL. No cytotoxicity was reported for the selected concentrations; however, the report stated that at the highest dose assayed, slight compound precipitation was observed.

B. UDS Assay: The UDS assay was conducted with 1.2, 6, 30, and 150 µg/mL of the test material. The choice of this dose range was based on the results of the preliminary cytotoxicity assay.

The authors did not report compound precipitation at the high dose. No evidence of a cytotoxic effect was observed at any dose, and the mean silver grains/nucleus for all doses were either comparable to or slightly lower than the control values. Representative results are presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors stated, "It is concluded that, under the given experimental conditions, no evidence of induction of DNA damage by G 30 027 or by its metabolites was obtained that could be interpreted as suggestive of mutagenic or carcinogenic properties of the substance."

B. A quality assurance statement was signed and dated May 10, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study is unacceptable for the following reasons:

1. Since the attachment period was prolonged (i.e., 24 hours), it is likely that the metabolic activity of the cells was reduced; hence, cells with less than adequate sensitivity were exposed to the test material. The recommended attachment period is 1.5 to 2 hours.[3]

2. The length of exposure of the hepatocytes to the test material (5 hours) may have been too short to induce a UDS response. The U.S. Environmental Protection Agency Gene-Tox Program[4] recommends an 18-hour exposure.

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[3] Mitchell, et al. (1983). Mutat. Res. 123(1983):363-410.

[4] Ibid.

Freshly isolated cells were suspended in Williams' Medium E (WME), counted, and dispensed (10^5 cells/mL) onto gelatinized coverslips in multi-well culture plates. The cultures were allowed to attach to the coverslips for 1.5-2.0 hours under unspecified conditions. Prior to use in the assay, attached cells were washed, refed fresh medium, and incubated overnight.

3. Preliminary Cytotoxicity Assay:

Cells were exposed to seven concentrations of the test material and the solvent control for 5 hours. Dosed cells were rinsed, stained with Trypan blue, and fixed, and the percentage of unstained cells in 100 scored hepatocytes was determined. The following criteria were used to evaluate the cytotoxicity results and to establish doses for the UDS assay: a sufficiently large number of cells must adhere to the coverslip, at least 25% of the cells must show viability upon examination by means of the vital-staining techniques, and a corresponding percentage of the cells must display normal morphological characteristics.

5. UDS Assay:

a. Treatment: Four preselected concentrations of the test material were evaluated in the UDS assay. Quadruplicate cultures per group were exposed to the test material doses, the negative control (untreated), the solvent control (ETOH), and 100 mM dimethylnitrosamine (DMN), the positive control, in the presence of 1 µCi/mL [^{3}H]-thymidine for 5 hours. Exposed cells were washed and fixed with ETOH/acetic acid (3:1) and the coverslips were mounted onto slides.

b. Preparation of Autoradiographs/Grain Development: The procedures and solutions used to develop the nuclear grains were not described; the report stated, however, that the exposure time was 6 hours. Autoradiographs were stained with hematoxylin-eosin. The report did not indicate whether the slides were coded.

c. Grain Counting: Nuclear grains of 150 cells for each of the dose and control groups were counted. The background count was determined in cell-free areas and the reported stated, "It was found to be negligibly low."

6. Evaluation Criteria: The assay was considered positive if the mean nuclear grain count was >2-fold higher than the negative control at any dose.

B. Protocol: A protocol was not presented.

7. CONCLUSIONS:

A. The primary rat hepatocyte unscheduled DNA synthesis (UDS) assay conducted with G 30 027 technical is unacceptable for the following reasons:

1. The combined 24-hour hepatocyte attachment period and 5-hour test compound exposure time caused a marked reduction in assay sensitivity as indicated by the less than adequate response of the positive control (see Reviewers' Discussion).

2. Cytoplasmic background grain counting was not performed.

3. Slides were not coded.

B. The study is unacceptable.

8. RECOMMENDATIONS:

The repeat assay should be performed in accordance with recommended procedures.[2]

Items 9 and 10—See footnote 2.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: G 30 027 was from batch No. P 210200 and had a purity of 98.2%. No information on the physical appearance, stability, or storage conditions was provided. The test material was dissolved in ethanol (ETOH).

2. Indicator Cells: Primary rat hepatocytes were collected from a male rat (Tif:RAIf, SPF); the method used to harvest the hepatocytes was not reported. The rat was obtained from CIBA-GEIGY Tierfarm, Sisseln.

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[1]Mitchell, A. D., Casciano, D. A., Meltz, M. L., Robinson, D. E., San, R. H. C., William, G. M., and Von Halle, E. S. Unscheduled DNA synthesis tests, a report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 123(1983):363-410.

[2]Only items appropriate to this DER have been included.

006718

Reviewed by: Sanford W. Bigelow, Ph.D. 5/6/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-04

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87048

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419
-and-
SRI International, 333 Ravenswood Ave., Mnelo Park, CA 94025 (Study No. LSC-1469)

TITLE OF REPORT: Disposition of Atrazine in the Rat (General Metabolism).

AUTHOR: G.R. Orr

REPORT ISSUED: October 23, 1987

CONCLUSIONS:

The distribution of atrazine in rats was found to be dose-dependent and independent of sex. Of the tissues studied, the red cells store the highest levels of atrazine, apparently through the covalent binding of a metabolite. In rats given a single oral dose of 100 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: red blood cells, heart, spleen, lung, liver, kidney, brain, gonads, pituitary, muscle, bone, fat, and plasma. In rats given repeated daily oral doses of 1 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: red blood cells, liver, spleen, kidney, lung, heart, pituitary, brain, gonads, muscle, bone, fat, and plasma. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red blood cell. The pattern of atrazine

tissue distribution for atrazine in this study is similar to that found in rats repeated exposed to atrazine (MRID Nos. 404313-05 and 404313-09).

The distribution of atrazine was reported to follow first-order kinetics. Two major relationships are found: (1) the whole body half-life, or $t_{1/2}$, and the volume of distribution, or V_d, are independent of the dose of atrazine and (2) the plasma concentration of atrazine or its metabolites are directly proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 31.3 hours (1.3 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

Excretion of atrazine in rats. About 95% of the atrazine administered orally is excreted within 7 days after cessation of exposure. The route of atrazine excretion was reported to be independent of the dose and sex of rat. About 75% of the atrazine is excreted through the urinary route whereas about 20% of the atrazine is eliminated in the feces. The elimination route for the remaining 5% was not reported. Also, the level of atrazine elimination by exhalation or through the skin (sweating) was not reported.

Summary. The whole body half-life of 1.30 days for atrazine is consistent with the observation that about 95% of the administered dose is eliminated within 7 days after exposure. The red cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the distribution and excretion of atrazine in male rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

II. MATERIALS:

A. Test Compound: Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine)

Description: Not provided in this summary report.
Batch #: Not provided in this summary report.
Purity: Not provided in this summary report for the nonradiolabeled compound.
Radiolabeling procedure:
All carbons in the triazine moiety of atrazine were replaced with carbon-14. The specific activity of the radiolabeled compound was 95.8 microCuries/mg in low dose experiments and 1.06 microCuries/mg in the high dose experiments. The purity of the radiolabeled test compound was reported to be ≥ 98% ascertained by two different thin-layer chromatography systems.

B. Test Animals:

Species: Rat
Strain: Sprague-Dawley CD
Age: Not provided in this report.
Weight (mean): 160-225g
Source: Charles River Breeding Laboratories, Portage, MI (refer to p. 59 of this study).

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study
(Atrazine Elimination and Distribution Experiment)

Test Group	Daily Oral Dose Given[a] (mg/kg)	Rats male	Rats female	Duration of Exposure
1 Control	0.0	2	2	none
2 Low	1.0	5	5	1 day
3 High	100.0	5	5	1 day
4 SubQ	1.0	5	5	15 days

[a] After the last oral dose was given, the urinary and fecal levels of radioactivity were measured for 7 days.

Animals were individually placed in metabolism cages for the collection of feces and urine. The collection of metabolite was conducted at SRI International. The samples were then shipped to the CIBA-GEIGY laboratory in Greensboro, NC for analysis.

B. Dose Method: The rats were allowed a one-week acclimation period prior to initiation of experimentation. Atrazine was given orally (via a stomach tube) to the rats as an active ingredient or as a radiolabeled active ingredient. The vehicle was 3% corn starch and 0.5% polysorbate 80 (v/v). The rats were allowed free access to animal feed (Purina) and tap water.

C. Statistics:

The following procedures were utilized in analyzing the numerical data:

One- and two-way analysis of variance (ANOVA) was used to assess the statistical significance of results between dose, treatment groups or sex. When appropriate, Dunnetts or Newman-Keuls t-tests were performed to assess differences between group means.

For generating the kinetic models, the excretion data was used. This evaluation was performed by I.W.F. Davidson of Bowman Gray School of Medicine. Additional kinetic parameters such as rate constants, half-life values, and alpha and beta distribution values were obtained with the use of the ESTRIP and PCNONLIN computer programs calculated by C.M. Metzler and D.L. Weiner (Statistical Consultants, Edgewood, KY).

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector from SRI International, the subcontracting laboratory where the metabolism of radiolabeled atrazine was studied. According to the statement, the Good Laboratory Practice methods were followed in this study. However, the analytical phase of the metabolism study was reported not meet the Good Laboratory Practices Requirements of 40 CFR Part 160 because: (1) "there was no QA [quality assurance] inspection of the analytical phase of the study" and (2) " there was no QA audit of [this] final report ABR-87042."

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this study.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Experimental Protocol: The procedure was conducted to assess the distribution (and elimination) of atrazine.

As shown in Table 1, three groups of rats (5 males and 5 females) were treated orally with atrazine. The first group received a single dose of ^{14}C-atrazine at 1 mg/kg; a second group were given a single dose of 100 mg/kg ^{14}C-atrazine; and a third group received daily doses of 1 mg/kg of nonradiolabeled atrazine for 14 days and on day 15, was given 1 mg/kg ^{14}C-atrazine. A control group received vehicle only.

Following the last dose of ^{14}C-atrazine in each group, the feces and urine were measured in each animal for 7 days. Following this, the rats were sacrificed and the urine, feces, and red blood cells, and the following selected tissues were analyzed for ^{14}C content (Figure 1).

FIGURE 1

Digestive system		**Cardiovascular**		**Neurological**			
	Tongue		Aorta*	X	Brain*+@		
	Salivary glands*	X	Heart*@		Peripheral nerve*#		
	Esophagus*		Bone marrow*#		Spinal cord (3 levels)*#		
	Stomach*		Lymph nodes*	X	Pituitary*		
	Duodenum*	X	Spleen@		Eyes (optic n.)*#		
	Jejunum*		Thymus*				
	Ileum*	X	Red blood cell		**Glandular**		
	Cecum*	**Urogenital**			Adrenal gland*		
	Colon*	X	Kidneys*+@		Exorbital lacrimal gland#		
	Rectum*		Bladder*	X	Mammary gland*#		
X	Liver *+@	X	Testes*+@		Parathyroids*++		
	Gall bladder*#		Epididymides		Thyroids*++		
	Pancreas*		Prostate	**Other tissues**			
Respiratory			Seminal vesicle	X	Bone (femur)*#		
	Trachea*#	X	Ovaries*+@	X	Muscle*#@		
X	Lung*@	X	Uterus*@		Skin*#		
	Nose^		Cervix		All gross lesions and masses*		
	Pharynx^		Fallopian tubes	X	Residual Carcass@		
	Larynx^			X	Fat@		
				X	Plasma (blood)@		

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
@ Required for determining distribution in metabolism studies.

V. RESULTS:

A. Distribution and Elimination of Atrazine and Its Metabolites

Five male and 5 female rats were used to assess the disposition and elimination of atrazine after acute or subchronic exposure. Table 2 shows that the total recovery of atrazine averaged 102.9% for the group given a single dose of 1 mg/kg ^{14}C-atrazine, 103.2% for the group of rats given a single dose of 100 mg/kg ^{14}C-atrazine, and 88.3% for the group of rats given a daily dose of 1 mg/kg atrazine followed by a single dose of 1 mg/kg ^{14}C-atrazine on day 15 (referred here as the subchronic group).

Concerning the elimination of atrazine or its metabolites, approximately 95% of the ^{14}C-label was excreted within 7 days of the last exposure (Table 2). In all 3 groups of rats, roughly 75% of the ^{14}C-label was excreted in the urine whereas about 20% of the ^{14}C-label was eliminated in the feces. Both discussion of other routes of elimination and the remaining 5% of the administered atrazine were not reported.

However, differences between dosage groups for tissue-borne ^{14}C-label were observed. A statistically significant decrease ($p < 0.05$) in the mean level of tissue-borne ^{14}C-label was found in those rats given a single dose of 100 mg/kg when compared to the group of rats who received a single dose of 1 mg/kg atrazine. Also, a statistically significant decrease ($p < 0.05$) in the mean level of tissue-borne ^{14}C-label was found in those rats subchronically treated with atrazine when compared to the group of rats who received a single dose of 1 mg/kg ^{14}C-atrazine. No differences were observed between sexes regarding ^{14}C-label in the urine, feces, and the tissues measured 7 days after exposure to ^{14}C-atrazine.

The red blood cells (RBC) had the highest levels of ^{14}C-label of all tissues studied (Table 3). The ratio of RBC binding of the ^{14}C-label was proportional to the dose administered, i.e., the concentration for the high dose single exposure group (100 mg/kg) was about 100 times that of the low dose single exposure group (1 mg/kg), and the tissue concentration of the subchronic group (1 mg/kg for 15 days) was the same (1.11 and 1.00) to that of the low dose group. The ratios, 1.11 and 1.00, also provide some indication that atrazine and its metabolites had not accumulated in the red blood cells or any other tissues

under this exposure regimen. This assertion is based on the observation that tissue concentrations were the same in the acute and subchronic exposure groups (Table 3).

The high concentration of ^{14}C-label reported in the red blood cell is discussed in further detail. The author suggests that ^{14}C-label binding in the red blood cell is the product of a covalent interaction between the triazine moiety of ^{14}C-atrazine and the cysteinal sulfhydryl groups in the rat hemoglobin macromolecule.

The remaining tissues listed in Table 3 show lower levels of ^{14}C-atrazine and its metabolites. Also, in these tissues, the ratio of ^{14}C-label binding was proportional lower than the administered dose, e.g., ^{14}C-label concentrations in the subchronic group were lower than that for the acute exposure group (Table 5). This finding provides evidence that atrazine or its metabolites appear not to accumulate in any tissues under this exposure regimen. However, cumulative binding of atrazine metabolites in RBCs after chronic exposure may occur.

Toxicokinetic modeling. The whole body half-life ($t_{1/2}$) of ^{14}C-label was 31.3 ± 2.8 hours (1.3 days) was calculated from the urinary excretion data. The author reported that the data best fits an open two-compartment toxicokinetic model. In addition, no statistically significant differences were reported between treatment groups or sex regarding the values for: alpha, beta, k_{10}, k_{12} and k_{21} or the whole body $t_{1/2}$ value.

Table 2
Distribution and Elimination of ^{14}C-Label (ppm) After 7 Days Following ^{14}C-Atrazine Treatment (taken from Table I)

Dose	1.0 mg/kg		100.0 mg/kg		1.0 mg/kg (subchronic)	
Sex (#)	Males(5)	Females(5)	Males(5)	Females(5)	Males(5)	Females(5)
Urine	0.77 ±0.01	0.77 ±0.02	77.27 ±1.67	79.86 ±2.16	0.67 ±0.04	0.62 ±0.09
Feces	0.18 ±0.01	0.19 ±0.01	21.34 ±0.55	17.85 ±0.71	0.19 ±0.01	0.17 ±0.01
Tissues	0.06 ±0.001	0.07 ±0.001	4.98 ±0.13	4.48 ±0.34	0.047 ±.002	0.046 ±0.002
Cage wash[a]	0.002 ±0.0004	0.003 ±0.001	0.33 ±0.08	0.29 ±0.11	0.005 ±0.001	0.006 ±0.001
Total	1.02 ±0.01	1.04 ±0.01	103.92 ±1.44	102.48 ±2.89	0.92 ±0.44	0.85 ±0.09
% Recovery	102.9 ±1.1		103.2 ±1.5		88.3 ±4.9	

[a] At sacrifice, the cages were washed with a water acetone mixture (1:1) and 10 ml aliquots were measured for radioactivity.

Table 3
Distribution and Elimination of ^{14}C-Label (ppm) After 7 Days Following ^{14}C-Atrazine Treatment
(taken from Table IV)

Dose	1.0 mg/kg		100.0 mg/kg		1.0 mg/kg (subchronic)	
Sex (#)	Males(5)	Females(5)	Males(5)	Females(5)	Males(5)	Females(5)
RBC	0.559	0.627	67.536	62.366	0.662	0.628
Kidney	0.229	0.263	6.936	6.990	0.155	0.140
Liver	0.247	0.498	7.378	7.468	0.204	0.212
Brain	0.166	0.162	5.210	4.580	0.076	0.070
Gonads	0.147	0.198	5.124	5.799	0.066	0.050
Heart	0.144	0.154	11.726	9.770	0.137	0.102
Spleen	0.136	0.148	10.748	12.563	0.156	0.169
Lung	0.115	0.134	9.229	9.128	0.111	0.132
Pituitary	0.080	0.081	4.126	4.220	0.088	0.074
Carcass	0.076	0.080	6.349	5.901	0.069	0.061
Muscle	0.060	0.067	4.080	3.637	0.044	0.041
Bone	0.044	0.047	3.476	3.625	0.042	0.038
Fat	0.015	0.011	1.245	1.320	0.014	0.009
Plasma	0.009	0.010	1.200	1.039	0.011	0.013
Mammaries	-	0.005	-	0.346	-	0.006
Uterus	-	0.033	-	3.743	-	0.047

V. **DISCUSSION:**

The distribution of atrazine in rats was found to be dose-dependent and independent of sex. Of the tissues studied, the red cells store the highest levels of atrazine, apparently through the covalent binding of a metabolite. In rats given a single oral dose of 100 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: red blood cells, heart, spleen, lung, liver, kidney, brain, gonads, pituitary, muscle, bone, fat, and plasma. In rats given repeated daily oral doses of 1 mg/kg atrazine, in decreasing order, the levels found in the following tissues were: red blood cells, liver, spleen, kidney, lung, heart, pituitary, brain, gonads, muscle, bone, fat, and plasma. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red blood cell. The pattern of atrazine tissue distribution for atrazine in this study is similar to that found in rats repeated exposed to atrazine (MRID Nos. 404313-05 and 404313-09).

The distribution of atrazine was reported to follow first-order kinetics. Two major relationships are found: (1) the whole body half-life, or $t_{1/2}$, and the volume of distribution, or V_d, are independent of the dose of atrazine and (2) the plasma concentration of atrazine or its metabolites are directly proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 31.3 hours (1.3 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

Excretion of atrazine in rats. About 95% of the atrazine administered orally is excreted within 7 days after cessation of exposure. The route of atrazine excretion was reported to be independent of the dose and sex of rat. About 75% of the atrazine is excreted through the urinary route whereas about 20% of the atrazine is eliminated in the feces. The elimination route for the remaining 5% was not reported. Also, the level of atrazine elimination by exhalation or through the skin (sweating) was not reported.

Summary. The whole body half-life of 1.30 days for atrazine is consistent with the observation that about 95% of the administered dose is eliminated within 7 days after exposure. The red cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the distribution and excretion of atrazine in male rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

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2Reviewed by: Sanford W. Bigelow, Ph.D. 5/6/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-05

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87087

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419
-and-
SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025 (Study No. LSC-1469)
-and-
Agrisearch Incorporated, 26 Water Street, Frederick, MD 21701 (Project No. 1271)

TITLE OF REPORT: Study of delta-^{14}C-Atrazine Dose/Response Relationship in the Rat (General Metabolism).

AUTHOR: B. Thede

REPORT ISSUED: October 23, 1987

CONCLUSIONS:

The distribution of atrazine in rats was found to be dose-dependent. Of the tissues studied, the red blood cells store the highest levels of atrazine, apparently through the covalent binding of a metabolite. In rats exposed to a dose of 100 mg/kg atrazine for 10 days, in decreasing order, the levels found in the following tissues were: red blood cell, liver, kidney, ovary, pituitary, brain, pectoral region of the mammaries. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red

blood cell. The pattern of atrazine tissue distribution found in this report was similar that found in male rats exposed to a similar dosage regimen (MRID No. 404313-09, Study No. ABR-85104).

The distribution of atrazine was reported to follow first-order kinetics. Two major relationships are found: (1) the whole body half-life, or $t_{1/2}$, and the volume of distribution, or V_d, are independent of the dose of atrazine and (2) the plasma concentration of atrazine and/or its metabolites are directly proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 38.6 hours (1.61 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

Summary. The whole body half-life for atrazine is 1.61 days. The red blood cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat, except perhaps for the red blood cell.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the disposition of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

II. MATERIALS:

A. Test Compound: Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine)

Description: Not provided in this summary report.
Batch #: S85-0653-J
Purity: 98.8% (expiration date - November, 1990)
Radiolabeling procedure:
All carbons in the triazine moiety of atrazine were replaced with carbon-14. The specific activity of the radiolabeled compound (reference CL-IX-77) was 95.8 microCuries/mg in low dose experiments and 1.06 microCuries/mg in the high dose experiments. The purity of the radiolabeled test compound was reported to be 97.9% ascertained by two different thin-layer chromatography systems.

B. Test Animals:

Species: Rat (female)
Strain: Sprague-Dawley CD
Age: Not provided in this report.
Weight (mean): 243.2g ± 2.7 SE (240-265g)
Source: Charles River Breeding Laboratories, Wilmington, MA

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study
(Atrazine Distribution Experiment)

Test Group	Daily Oral Dose Given (mg/kg)	Rats (female)	Duration of Exposure (days)
1 Control	0	2	10
2 Low1 (LDT1)	1.0	2	10
3 Mid1 (MDT1)	3.0	2	10
4 Mid2 (MDT2)	7.0	2	10
5 Low3 (MDT3)	10.0	2	10
6 Mid4 (MDT4)	50.0	2	10
7 High (HDT1)	100.0	2	10

a After the last oral dose was given, the urinary and fecal levels of radioactivity were measured for 7 days. Animals were individually placed in metabolism cages for the collection of feces and urine. The collection of metabolite was conducted at SRI International. The samples were then shipped to the CIBA-GEIGY laboratory in Greensboro, NC for analysis.

B. Dose Method: The rats were allowed a one-week acclimation period prior to initiation of experimentation. Atrazine was given orally (via a stomach tube) to the rats as an active ingredient or as a radiolabeled active ingredient. The vehicle was 3% corn starch and 0.5% polysorbate 80 (v/v). The rats were allowed free access to animal feed (Purina) and tap water.

Table 4
Tissue Levels of ^{14}C-Label (ppm) at Sacrifice
(taken from Table X)

Dose	1 mg/kg		3 mg/kg		7 mg/kg		10 mg/kg		50 mg/kg		100 mg/kg	
Rat #:	R5062	R5063	R5064	R5065	R5066	R5067	R5068	R5069	R5070	R5071	R5072	R5073
Hour of Sacrifice:	3	72	72	3	3	72	3	72	3	72	3	72
Tissue:												
Liver	2.97	2.33	5.40	8.06	16.21	8.05	20.86	12.37	54.87	32.58	102.37	55.8
Pituitary	1.18	0.50	1.67	3.54	7.32	3.24	9.36	4.49	36.91	18.18	71.68	33.9
Ovary	1.14	0.48	1.59	3.62	7.28	2.81	9.08	4.69	36.30	17.42	76.39	33.0
Brain	0.39	0.24	0.90	1.57	3.24	1.55	4.12	2.04	14.59	8.99	30.25	11.8
Kidney	1.36	0.61	1.64	3.54	6.91	3.35	9.88	4.70	29.31	16.73	78.64	26.1
Mammaries:												
Pectoral	0.13	0.05	0.38	0.46	0.52	0.24	1.24	0.75	4.41	3.32	6.30	7.3
Inguinal	0.06	0.05	0.15	0.19	0.79	0.11	0.54	0.65	4.06	2.29	4.77	0.3

V. DISCUSSION

The distribution of atrazine in rats was found to be dose-dependent. Of the tissues studied, the red blood cells store the highest levels of atrazine, apparently through the covalent binding of a metabolite. In rats exposed to a dose of 100 mg/kg atrazine for 10 days, in decreasing order, the levels found in the following tissues were: red blood cell, liver, kidney, ovary, pituitary, brain, pectoral region of the mammaries. Under the exposure regimen used in this study, atrazine does not accumulate in the tissues of the rat, except perhaps for the red blood cell. The pattern of atrazine tissue distribution found in this report was similar that found in male rats exposed to a similar dosage regimen (MRID No. 404313-09, Study No. ABR-85104).

The distribution of atrazine was reported to follow first-order kinetics. Two major relationships are found: (1) the whole body half-life, or $t_{1/2}$, and the volume of distribution, or V_d, are independent of the dose of atrazine and (2) the plasma concentration of atrazine and/or its metabolites are directly proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 38.6 hours (1.61 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

Summary. The whole body half-life for atrazine is 1.61 days. The red blood cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat, except perhaps for the red blood cell.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the disposition of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

Reviewed by: Sanford W. Bigelow, Ph.D.
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D.
Section VI, Toxicology Branch (TS-769C)

ADDENDUM TO THE DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-06

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87115

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: Characterization and Identification of Atrazine Metabolites From Rat Urine (General Metabolism).

AUTHOR: B.J. Miles

REPORT ISSUED: November 17, 1987

CONCLUSIONS:

After further review of MRID No. 404313-06 and the data evaluation report on MRID No. 404313-06, it was found that the major urinary metabolites in the female rat are chlorinated triazines, not hydroxylated triazines as stated obstensibly in MRID No. 404313-06. The registrant states that the hydroxylated metabolites of atrazine are artifacts of the procedure used to isolate the metabolites. Therefore, the major urinary metabolites of atrazine in female rats reported in MRID No. 404313-06 are:

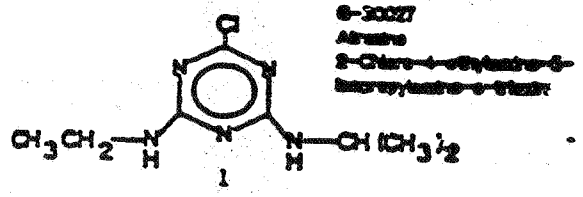
- 2-chloro-4-amino-6-isopropylamino-s-triazine (13),
- 2-chloro-4-ethylamino-6-amino-s-triazine (14), and
- 2-chloro-4,6-diamino-s-triazine (15).

The molecular structures of the above atrazine metabolites are shown in Figure 1 (the numbers in Figure 1 correspond to those associated with the above metabolites). Of the metabolites listed above, 2-chloro-4,6-diamino-s-triazine (15) is reported to be the major urinary metabolite. The identification of the metabolites above indicates that N-dealkylation is the major metabolic pathway for atrazine in female rats.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the identity of urinary metabolites of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported, i.e., the urinary and fecal metabolites of atrazine in male rats and the fecal metabolites of atrazine in females must be identified.

PC1/reports/atramet1.006

FIGURE I. CHEMICAL NAMES AND STRUCTURES



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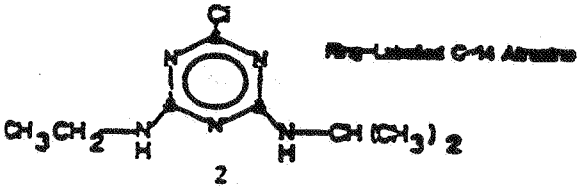

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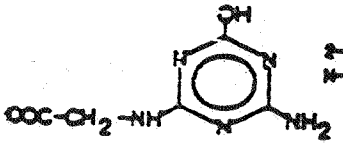

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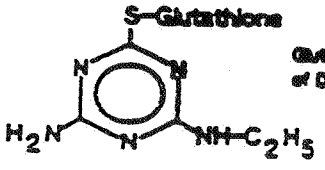

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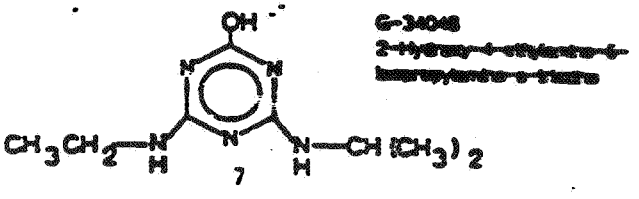

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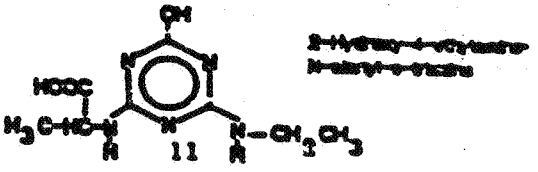

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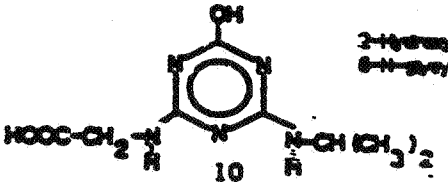

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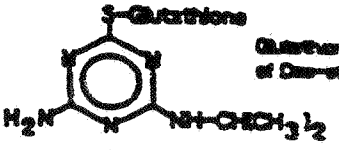

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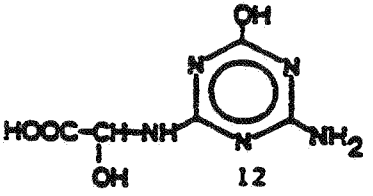

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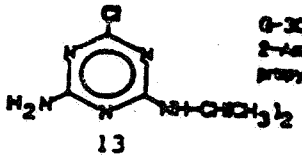

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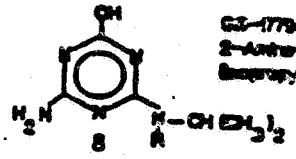

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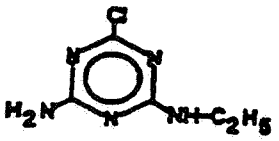

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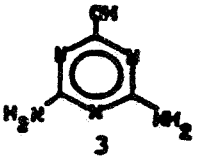

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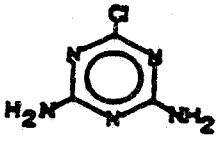

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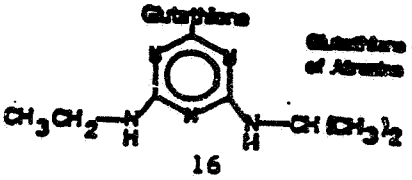

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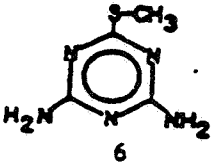

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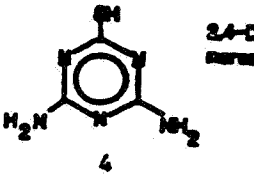

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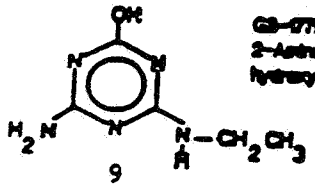

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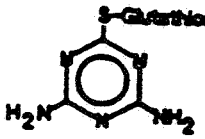

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FIGURE I. CHEMICAL NAMES AND STRUCTURES (Cont.)

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Reviewed by: Sanford W. Bigelow, Ph.D. 5/6/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-06

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87115

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: Characterization and Identification of Atrazine Metabolites From Rat Urine (General Metabolism).

AUTHOR: B.J. Miles

REPORT ISSUED: November 17, 1987

CONCLUSIONS:

The characterization and identification of a number of atrazine metabolites in the female rat was reported in this study. To this end, two experiments were conducted with the use of two groups of rats.

The data reported in this study indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alkyl substituents of atrazine appears to be a minor and secondary metabolic route.

The elimination of atrazine in female rats was also reported in this study. The urinary route accounted for 47.4% of the elimination of atrazine and/or its metabolites whereas 49.3% was eliminated via the fecal route. The tissues contained 5.75% of the atrazine and/or its metabolites while the blood contained the remaining 1.4%. This pattern of excretion differs from male or female rats given repeated oral doses of atrazine, i.e., single oral exposure results in about 50:50 urinary:fecal excretion whereas repeated oral exposure results in about about 75:25 urinary:fecal excretion (see MRID Nos. 404313-05 and 404313-09 for more details). The amount of atrazine and/or its metabolites eliminated via exhalation was not reported. A recovery of 103.78% of the administered radiolabeled atrazine was achieved. The majority of atrazine and/or its metabolites was reported to be excreted via the urine and feces.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the identity of urinary metabolites of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported, i.e., the urinary and fecal metabolites of atrazine in male rats and the fecal metabolites of atrazine in females must be identified.

II. MATERIALS:

A. Test Compound: Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine)

Description: Not provided in this report.
Batch #: Not provided in this report.
Purity: Not provided in this report for the nonradiolabeled compound.
Radiolabeling procedure:
All carbons in the triazine moiety of atrazine were replaced with carbon-14. The specific activity of the radiolabeled compound was 1.0 microCurie/mg. The purity of the radiolabeled test compound was reported to be ≥ 97%.

B. Test Animals:

Species: Rat (female)
Strain: Sprague-Dawley
Age: Not provided in this report.
Weight (mean): about 0.2 kg
Source: Harlan Sprague-Dawley, Indianapolis, IN

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study
(Atrazine Metabolism Experiment)

Test Group	Daily Oral Dose Given (mg/kg)	Rats (female)	Duration of Exposure (day)
1 High	100.0	5	1
2 Mid	16.2 - 19.6	8	1

[a] After the last oral dose was given, the urinary and fecal levels of radioactivity were measured for 24 hours. Animals were individually placed in metabolism cages for the collection of urine.

B. Dose Method: The rats were allowed a 5-day acclimation period prior to initiation of experimentation. Atrazine was given orally (via a stomach tube) to the rats as an active ingredient or as a radiolabeled active ingredient. The vehicle was 1% methyl carboxymethyl cellulose and Hi-Sil-233 brand of powdered silica used to suspend the atrazine in solution. The rats were allowed free access to animal feed (Purina) and deionized water.

C. Statistics:

No statistical procedures were used in this study.

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector from the registrant, the laboratory where the metabolism of radiolabeled atrazine was studied. According to the statement, the Good Laboratory Practice methods were followed in this study. However, this metabolism study was reported not meet the Good Laboratory Practices Requirements of 40 CFR Part 160 because:

(1) "A complete set of biological phase SOPs have not been established.

(2) There was no QA inspection of the study because the QAU was not a fully functional unit at the time the study was conducted.

(3) There was no QA audit of the final report ABR-87115."

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this summary report.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Experimental Protocol: This experiment was conducted to identify the atrazine metabolites in two groups of rats.

As shown in Table 1, one group of female rats was given a single dose of atrazine in an effort to produce sufficient levels of urinary metabolites of atrazine for identification. Five adult female Sprague-Dawley rats (about 0.2 kg) were administered 100 mg/kg ^{14}C-atrazine. Samples of urine and feces were obtained at 24, 48, and 72 hours. After taking samples for 72 hours, the rats were sacrificed and 5 ml of blood and the liver were obtained.

In another group of animals, 8 rats were given a single oral exposure of 16.18 - 19.64 mg/kg ^{14}C-atrazine. Urinary metabolites were collected over a 24-hour period following treatment. The metabolites of atrazine were isolated and identified by the following series of analytical chemistry steps:

(1) charcoal cleanup,

(2) C_{18} Bond-Elut separation,

(3) Aminex A-4 cation exchange column chromatography,

(4) Aminex A-25 anion exchange column chromatography or PRP-1 (reverse-phase) HPLC, and finally

(5) confirmation by comparing to the infrared spectra and mass spectra of authentic synthesized standards.

V. RESULTS:

A. The In Vivo Metabolism of Atrazine.

To examine the metabolism of atrazine in rats, 100 mg/kg of ^{14}C-atrazine was given to rats and the ^{14}C-labeled metabolites were isolated and identified. A recovery of 103.78% of the total radioactivity was achieved. The urinary route accounted for 47.4% of the elimination whereas 49.3% of the ^{14}C-label was eliminated via the fecal route. The tissues contained 5.75% of the ^{14}C-label while the blood contained the remaining 1.4% of the ^{14}C-label. The amount of ^{14}C-label eliminated via exhalation was not reported.

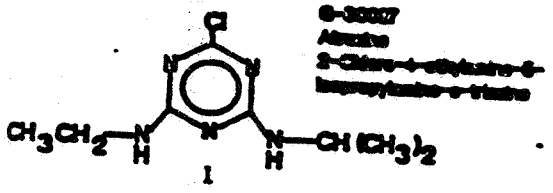
The molecular structures of the urinary metabolites obtained from the first group rats were unattainable, so a second group of 8 rats were given 16.18-19.64 mg/kg ^{14}C-atrazine. The metabolites were collected within the 0 to 24 hour time period after exposure. The urine was freeze dried. Metabolites were then dissolved in a small amount of water that was acidified with HCl to pH 3.0 and separated with an amino acid analyzer (to detect the amino acid residues of glutathione) coupled with a cation exchange column.

A total of 19 radioactive peaks were detected, three of which were identified as metabolites by comparison of the infrared and mass spectra. The identity of two other metabolites was postulated based on additional mass spectral information. The molecular structures of some of the atrazine metabolites are shown in Figure 1 and the numbers in this figure correspond to the metabolites discussed in the text. Only four of these metabolites were identified and were reported, they were:

- 2-hydroxy-atrazine (7),
- 2-hydroxy-4-amino-6-isopropylamino-s-triazine (8),
- 2-hydroxy-4-ethylamino-6-amino-s-triazine (14), and
- 2-hydroxy-4,6-diamino-s-triazine (3).

The identification of the four metabolites above indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Because several other minor metabolites that possess omega-carboxyl moieties were identified (5, 10, 11, 12),

FIGURE I. CHEMICAL NAMES AND STRUCTURES



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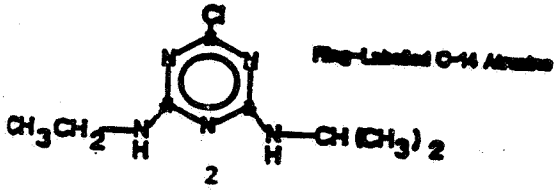

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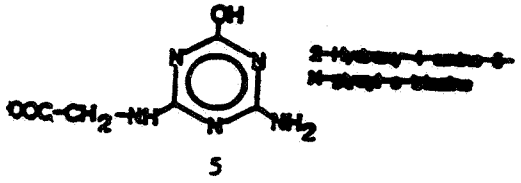

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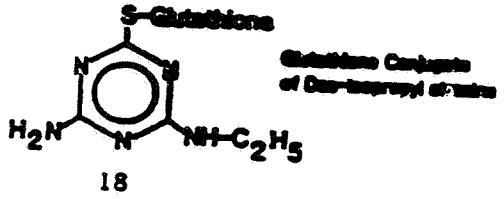

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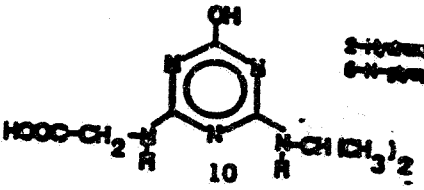

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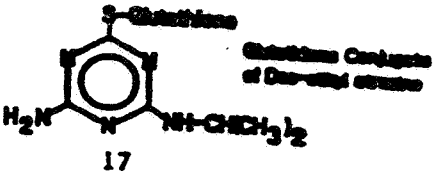

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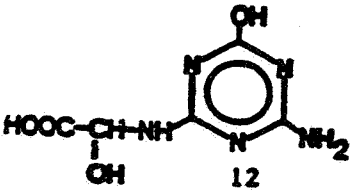

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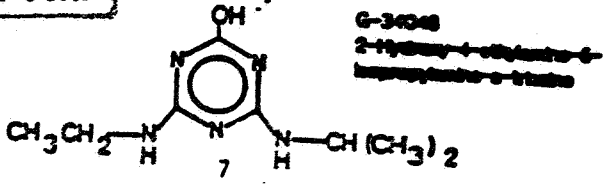

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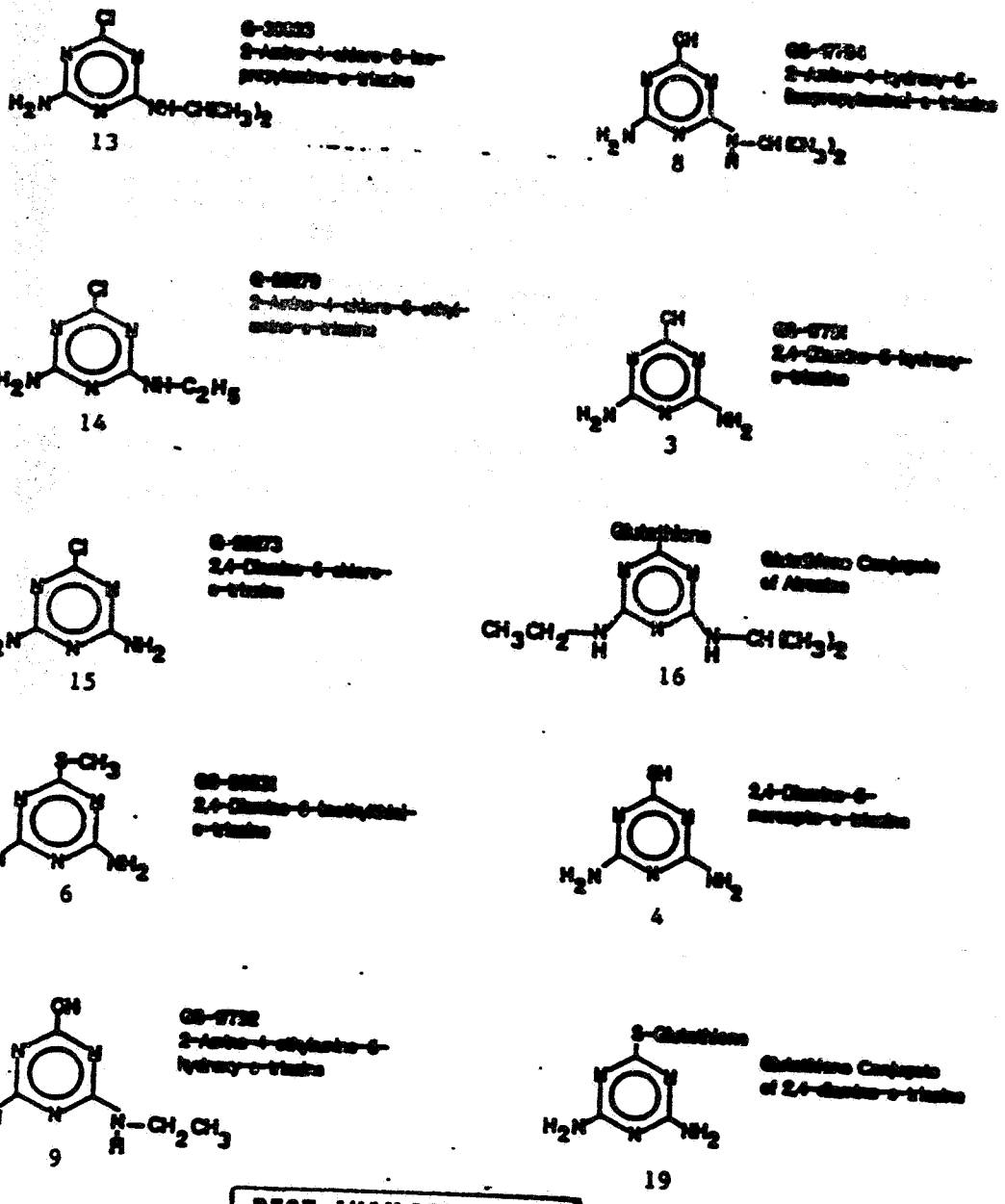

FIGURE I. CHEMICAL NAMES AND STRUCTURES (Cont.)

oxidation of the terminal methyl moieties in the alkyl substituents appears to be a minor and secondary metabolic route.

B. The In Vitro Metabolism of Atrazine.

The author of this study offers the results of a published study on atrazine metabolism performed by Dauterman and Muecke (1974. Pesticide Biochemistry and Physiology 4:212-219) in an effort to account for the covalent binding of atrazine in RBCs.

The method published by Dauterman and Muecke is reported as the following steps. Radiolabeled atrazine was incubated with rat liver microsomes with or without the addition of the metabolic cofactors, glutathione and NADPH. Six metabolites were identified by chromatography against synthetic standards. The results corroborate the findings in the in vivo experiment that N-dealkylation is the major metabolic pathway. Also, the isopropyl moiety is hydrolyzed more easily than the ethyl substituent. Conjugation with glutathione was found to occur with most of the atrazine metabolites previously discussed when cytosolic cell fractions were included in the in vitro reactions.

Covalent binding in RBCs. The author argues that the glutathione-containing metabolites of atrazine may be catalyzed by a "carbon-sulfur lyase," an enzyme that cleaves the glutathione residue and leaves a thiol group on the atrazine metabolite. However, the author has not presented evidence whether lyase is present in red blood cells.

V. **DISCUSSION:**

The characterization and identification of a number of atrazine metabolites in the female rat was reported in this study. To this end, two experiments were conducted with the use of two groups of rats.

The data reported in this study indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alkyl substituents of atrazine appears to be a minor and secondary metabolic route.

The elimination of atrazine in female rats was also reported in this study. The urinary route accounted for 47.4% of the elimination of atrazine and/or its metabolites whereas 49.3% was eliminated via the fecal route. The tissues contained 5.75% of the atrazine and/or its metabolites while the blood contained the remaining 1.4%. This pattern of excretion differs from male or female rats given repeated oral doses of atrazine, i.e., single oral exposure results in about 50:50 urinary:fecal excretion whereas repeated oral exposure results in about about 75:25 urinary:fecal excretion (see MRID Nos. 404313-05 and 404313-09 for more details). The amount of atrazine and/or its metabolites eliminated via exhalation was not reported. A recovery of 103.78% of the administered radiolabeled atrazine was achieved. The majority of atrazine and/or its metabolites was reported to be excreted via the urine and feces.

Classification: **Acceptable:** This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the identity of urinary metabolites of atrazine in female rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported, i.e., the urinary and fecal metabolites of atrazine in male rats and the fecal metabolites of atrazine in females must be identified.

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Reviewed by: Sanford W. Bigelow, Ph.D. 5/5/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404313-09

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-85104

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: Metabolism of ^{14}C-Atrazine in Orally Dosed Rats (General Metabolism).

AUTHOR: B.J. Simoneaux

REPORT ISSUED: December 6, 1985

CONCLUSIONS:

This report is a balance study of the disposition of atrazine in male rats repeatedly orally exposed to this agent. Atrazine appears to be rapidly excreted in the rat under these exposure conditions. About 95% of the administered dose is eliminated within 18 days after the last atrazine exposure. The urinary route accounted for about 70% of the elimination whereas about 25% was eliminated via the fecal route. The RBCs store the highest levels followed by the liver, kidney and brain. Under these exposure conditions, atrazine does not accumulate in the rat. The total recovery of administered radiolabeled atrazine for the high and low dose groups was 93.4% and 103.9%, respectively.

Atrazine appears to be rapidly excreted in the rat under these exposure conditions. About 95% of the administered dose is eliminated within 18 days after the last atrazine exposure. For the low and high dose groups of rats, respectively, the urinary route accounted for 72.7% and 67.2% of the elimination while 27.8% and 23.9% of the atrazine and/or its metabolites were eliminated via the fecal route. Elimination of atrazine and/or its metabolites by way of exhalation was not monitored or reported.

The tissues contained the remaining amount of the atrazine and/or its metabolites. The peak tissue levels in the low dose group occurred at 10 days whereas the peak levels in the high dose group was reported at 8 days. The highest tissue levels in the low dose group (0.1 mg/rat) were found at 10 days in the RBC followed by liver, kidney and brain. In decreasing order, the highest tissue levels of atrazine in the high dose group of rats (1.0 mg/rat) at 8 days were: RBC, liver, kidney and brain. In general, 10 days after the last dose of atrazine (at the 18-day sacrifice), the RBCs, liver, kidney and brain had minimal levels (about 1%) of atrazine and/or its metabolites remaining. Under these exposure conditions, atrazine does not accumulate in these tissues in rats repeatedly exposed to atrazine. The pattern of atrazine tissue distribution found in this report was similar that found in female rats exposed to a similar dosage regimen (MRID No. 404313-05, Study No. ABR-87087).

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the distribution and excretion of atrazine in male rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

II. MATERIALS:

A. Test Compound:

Description: Atrazine
Batch #: Not reported in this study
Purity: Not provided in this summary report for the nonradiolabeled compound.
Radiolabeling procedure:
All carbons in the triazine moiety of atrazine were replaced with carbon-14. The specific activity of the radiolabeled compound were 13.5 microCuries/mg and 12.9 microCuries/mg for the low and high dose groups, respectively. The purity of the radiolabeled test compound was reported to be $\geq$ 97.5% ascertained by a thin-layer chromatography system.

B. Test Animals:

Species: Rat (male)
Strain: Harlan Sprague-Dawley
Age: Not provided in this report.
Weight (mean): 250g
Source: Harlan Madison, WI

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study
(Atrazine Metabolism Experiment)

Test Group	Daily Oral Dose Given[a] (mg/kg)	Rats (male)	Day of Sacrifice	Duration of Exposure (days)
2 Low	0.4	3	5	4
Low	0.4	3	7	7
Low	0.4	3	9	7
Low	0.4	3	10	7
Low	0.4	3	14	7
Low	0.4	3	18	7
3 High	4.0	3	5	4
High	4.0	3	7	7
High	4.0	3	9	7
High	4.0	3	10	7
High	4.0	3	14	7
High	4.0	3	18	7

[a] After the last oral dose was given, the urinary and fecal levels of radioactivity were measured at 24-hour intervals in the group of rats exposed for 18 days. Animals were individually placed in metabolism cages for the collection of feces and urine. There was no control group.

B. Dose Method: Atrazine was given orally (via a stomach tube) to the rats as a radiolabeled active ingredient. The 250 g rats were given 0.1 mg/rat (low dose) or 1.0 mg/rat (high dose). The vehicle was in the aqueous Carbowax-200 (PEG 200) formulation (0.3 ml ethanol:0.2 ml water:0.5 ml PEG 200). The rats were allowed free access to animal feed and tap water.

C. Statistics:

The following procedure was utilized in analyzing the numerical data:

The SOP method of Wolf and Sumner, AG-276, "Statistical methods in the measurement of radioactivity" were used to calculate ppm-equivalents of the ^{14}C-label obtained from the rats.

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector from the registrant, the laboratory where the metabolism of radiolabeled atrazine was studied. According to the statement, the Good Laboratory Practice methods were followed in this study.

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this study.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Experimental Protocol: The procedure was conducted to assess the metabolism of atrazine.

Three male rats in each group were repeatedly dosed and then sacrificed 5, 7, 9, 10, 14, and 18 days after dosing was initiated (for details, see Table 1). Urine and feces were collected for analysis from the rats exposed for the 18-day period. The rats were sacrificed and the following selected tissues were analyzed for ^{14}C content (Figure 1).

FIGURE 1

Digestive system		**Cardiovascular**		**Neurological**	
	Tongue		Aorta*	X	Brain*+@
	Salivary glands*		Heart*@		Peripheral nerve*#
	Esophagus*		Bone marrow*#		Spinal cord (3 levels)*#
X	Stomach*		Lymph nodes*		Pituitary*
X	Duodenum*		Spleen@		Eyes (optic n.)*#
X	Jejunum*		Thymus*		
X	Ileum*	X	Red blood cell	**Glandular**	
	Cecum*	**Urogenital**			Adrenal gland*
X	Colon*	X	Kidneys*+@		Exorbital lacrimal gland#
	Rectum*		Bladder*		Mammary gland*#
X	Liver *+@		Testes*+@		Parathyroids*++
	Gall bladder*#		Epididymides		Thyroids*++
	Pancreas*		Prostate	**Other tissues**	
Respiratory			Seminal vesicle		Bone (femur)*#
	Trachea*#		Ovaries*+@	X	Muscle*#@
	Lung*@		Uterus*@		Skin*#
	Nose^		Cervix		All gross lesions and masses*
	Pharynx^		Fallopian tubes	X	Residual Carcass@
	Larynx^			X	Fat@
				X	Plasma (blood)@

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
@ Required for determining distribution in metabolism studies.

[FIFRA Subdivision F test guidelines §85-1 (e)(3)(i) require that, in addition to the tissues listed in Figure 1 above, the levels of atrazine or its metabolites shall be measured in the testes, heart, lung, spleen and uterus.]

V. RESULTS:

B. The Metabolism of Atrazine

To examine the metabolism of atrazine in rats, two doses were employed, 0.4 and 4.0 mg/kg of ^{14}C-atrazine was given to rats and the ^{14}C-label was measured in selected tissues and in the rats exposed for 18-days, urinary and fecal levels of ^{14}C-label were monitored. A recovery of 103.9% and 93.4% was found for the low and high dose groups, respectively. For the low and high dose groups, respectively, the urinary route accounted for 72.74% and 67.2% of the elimination whereas 27.79% and 23.92% of the ^{14}C-label was eliminated via the fecal route. The author reports that about 95% of the administered dose is eliminated within 48 hours after the last exposure.

The tissues contained the remainder of the ^{14}C-label (Tables 2 and 3). The highest tissue levels in the low dose group (0.1 mg/rat) were found at 10 days in the RBC (1.95 ppm) followed by liver (1.10 ppm), kidney (0.74 ppm) and brain (0.38 ppm) and are listed in Table 2. The highest tissue levels of ^{14}C-label, in decreasing order, in the high dose group of rats at 8 days were found as such: RBC (21.66 ppm), liver (6.40 ppm), kidney (5.28 ppm) and brain (2.48 ppm). In general, 10 days after the last dose of ^{14}C-atrazine (at the 18-day sacrifice), the RBCs, liver, kidney and brain had minimal levels (about 1%) of ^{14}C-label remaining. The remaining tissues had lower levels of ^{14}C-label at 8 or 10 days and lower levels remaining at 18 days. The peak tissue levels in the low dose group occurred at 10 day whereas the peak levels in the high dose group was reported at 8 days.

As percentage of administered dose (Table 3), the muscle had the highest levels followed by the liver and RBC. Percentage of tissue levels were highest in those rats sacrificed 4 days after initial atrazine exposure (Table 3).

Table 2
Tissue Levels of ^{14}C-Label (ppm) Remaining After Sacrifice
(taken from Table IV)

	Time of Sacrifice (Days)					
0.1 mg/rat	4	6	8	10	14	18
Plasma	0.06	0.05	0.06	0.04	0.01	0.01
RBC	1.11	1.18	1.63	1.95	1.31	1.53
Fat	0.04	0.04	0.04	0.05	0.05	0.03
Brain	0.29	0.30	0.39	0.38	0.27	0.24
Muscle	0.13	0.13	0.15	0.15	0.12	0.11
Kidney	0.67	0.63	0.74	0.71	0.32	0.23
Liver	0.88	0.91	1.06	1.10	0.56	0.40
Stomach	0.20	0.66	0.21	0.18	0.10	0.10
Small Intestine	0.21	0.24	0.26	0.15	0.06	0.09
Large Intestine	6.17	0.25	0.20	0.16	0.07	0.09
Large Intestinal Content	0.80	0.87	0.64	0.30	0.11	0.12
1.0 mg/rat						
Plasma	0.55	0.82	1.02	0.37	0.14	0.07
RBC	9.30	16.34	21.66	17.17	15.50	13.78
Fat	0.21	0.28	0.43	0.23	0.15	0.19
Brain	1.23	2.10	2.48	1.76	1.39	1.14
Muscle	0.77	1.27	1.56	0.91	0.83	0.75
Kidney	3.09	4.49	5.28	3.41	1.92	1.26
Liver	4.26	5.48	6.40	4.48	2.87	1.80
Stomach	1.46	2.01	1.96	0.52	0.32	0.15
Small Intestine	1.44	1.87	2.22	0.75	0.32	0.22
Large Intestine	1.28	1.56	1.86	0.97	0.60	0.56
Large Intestinal Content	9.15	12.27	11.94	1.28	7.97	0.22

Table 3
Percent of Dose of ^{14}C-Label Remaining After Sacrifice
(taken from Table III)

	Time of Sacrifice (Days)					
0.1 mg/rat	4	6	8	10	14	18
Plasma	0.15	0.08	0.10	0.05	0.02	0.01
RBC	1.60	1.28	1.39	1.38	1.08	1.11
Fat	0.26	0.20	0.09	0.37	0.16	0.09
Brain	0.12	0.08	0.09	0.08	0.06	0.05
Muscle	3.66	2.65	2.51	2.05	1.81	1.55
Kidney	0.34	0.26	0.23	0.17	0.10	0.06
Liver	2.55	1.94	1.70	1.28	0.85	0.43
Stomach	0.15	0.35	0.10	0.05	0.03	0.02
Small Intestine	0.15	0.18	0.22	0.07	0.03	0.02
Large Intestine	0.04	0.06	0.03	0.02	0.01	0.01
Large Intestinal Content	0.25	0.29	0.13	0.07	0.02	0.02
1.0 mg/rat						
Plasma	0.15	0.13	0.12	0.05	0.02	0.01
RBC	1.48	1.51	1.46	1.38	1.08	0.94
Fat	0.16	0.12	0.13	0.08	0.04	0.06
Brain	0.05	0.06	0.04	0.04	0.03	0.02
Muscle	2.42	2.23	2.04	1.41	1.12	1.00
Kidney	0.18	0.13	0.11	0.09	0.05	0.03
Liver	1.27	0.87	0.72	0.72	0.42	0.25
Stomach	0.08	0.06	0.10	0.03	0.02	0.01
Small Intestine	0.19	0.10	0.09	0.05	0.02	0.01
Large Intestine	0.04	0.02	0.02	0.02	0.01	0.01
Large Intestinal Content	0.38	0.36	0.13	0.04	0.18	0.01

VI. DISCUSSION

This report is a balance study of the disposition of atrazine in male rats repeatedly orally exposed to this agent. Atrazine appears to be rapidly excreted in the rat under these exposure conditions. About 95% of the administered dose is eliminated within 18 days after the last atrazine exposure. The urinary route accounted for about 70% of the elimination whereas about 25% was eliminated via the fecal route. The RBCs store the highest levels followed by the liver, kidney and brain. Under these exposure conditions, atrazine does not accumulate in the rat. The total recovery of administered radiolabeled atrazine for the high and low dose groups was 93.4% and 103.9%, respectively.

Atrazine appears to be rapidly excreted in the rat under these exposure conditions. About 95% of the administered dose is eliminated within 18 days after the last atrazine exposure. For the low and high dose groups of rats, respectively, the urinary route accounted for 72.7% and 67.2% of the elimination while 27.8% and 23.9% of the atrazine and/or its metabolites were eliminated via the fecal route. Elimination of atrazine and/or its metabolites by way of exhalation was not monitored or reported.

The tissues contained the remaining amount of the atrazine and/or its metabolites. The peak tissue levels in the low dose group occurred at 10 days whereas the peak levels in the high dose group was reported at 8 days. The highest tissue levels in the low dose group (0.1 mg/rat) were found at 10 days in the RBC followed by liver, kidney and brain. In decreasing order, the highest tissue levels of atrazine in the high dose group of rats (1.0 mg/rat) at 8 days were: RBC, liver, kidney and brain. In general, 10 days after the last dose of atrazine (at the 18-day sacrifice), the RBCs, liver, kidney and brain had minimal levels (about 1%) of atrazine and/or its metabolites remaining. Under these exposure conditions, atrazine does not accumulate in these tissues in rats repeatedly exposed to atrazine. The pattern of atrazine tissue distribution found in this report was similar that found in female rats exposed to a similar dosage regimen (MRID No. 404313-05, Study No. ABR-87087).

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting the distribution and excretion of atrazine in male rats. However, all of the data requirements for metabolism studies set forth in §85-1 have not been reported.

Reviewed by: Sanford W. Bigelow, Ph.D.
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D.
Section VI, Toxicology Branch (TS-769C)

006937

ADDENDUM TO THE DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404375-01

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87116

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: A Summary of the Disposition, Kinetics and Metabolism of Atrazine in the Rat (General Metabolism).

AUTHOR: G.R. Orr

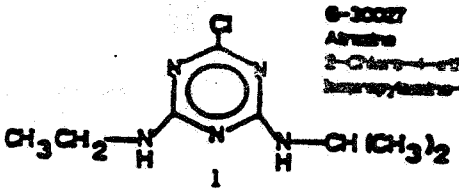
REPORT ISSUED: November 17, 1987

CONCLUSIONS:

After further review of MRID No. 404375-01 and the data evaluation report on MRID No. 404375-01, it was found that the major urinary metabolites in the female rat are chlorinated triazines, not hydroxylated triazines as stated superficially in MRID No. 404375-01. The registrant states that the hydroxylated metabolites of atrazine are artifacts of the procedure used to isolate the metabolites. The major urinary metabolite of atrazine in female rats reported in MRID No. 404375-01 is 2-chloro-4,6-diamino-s-triazine (15). The molecular structure of this atrazine metabolite is shown in Figure 1 (the number in Figure 1 correspond to number '15' with the above metabolites). The identification of the metabolites above indicates that N-dealkylation is the major metabolic pathway for atrazine in female rats.

Classification: Acceptable: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting (1) the identity of urinary metabolites of atrazine in female rats as well as (2) the distribution and excretion of atrazine in male and female rats. However, all of the data requirements for metabolism studies set forth in Subdivision F §85-1 have not been reported, i.e., (a) the urinary and fecal metabolites of atrazine in male rats and (b) the fecal metabolites of atrazine in females must be identified to satisfy completely the §85-1 data reporting requirements for the metabolism of atrazine in the rat.

FIGURE I. CHEMICAL NAMES AND STRUCTURES



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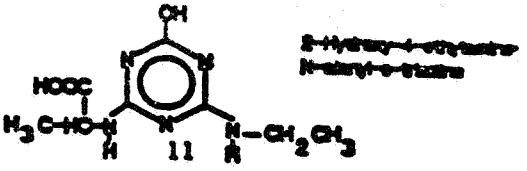

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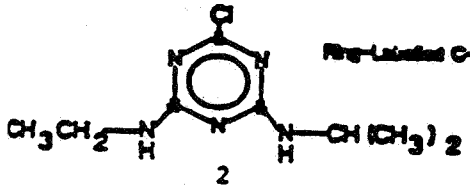

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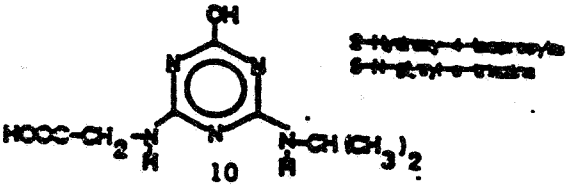

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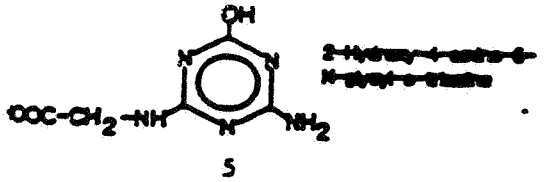

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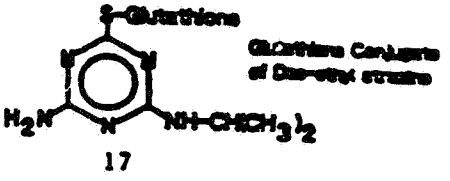

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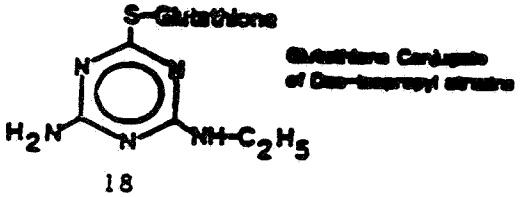

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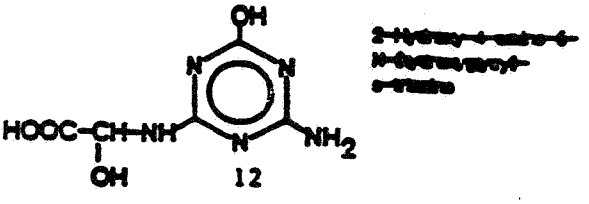

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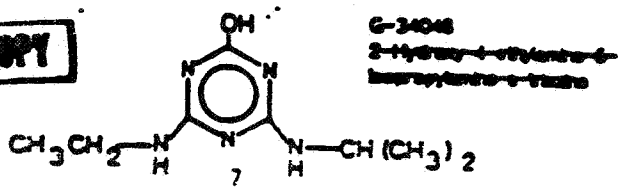

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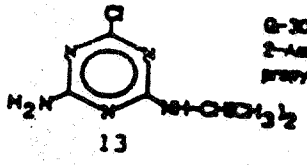

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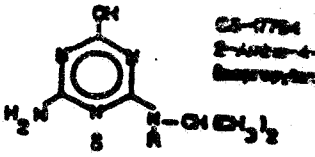

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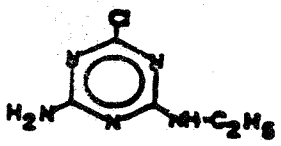

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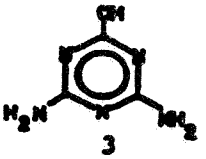

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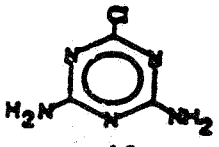

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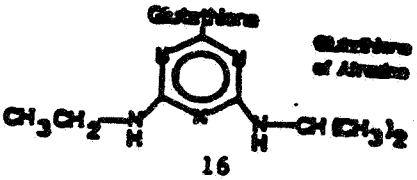

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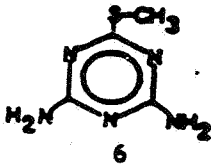

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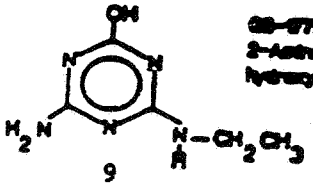

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FIGURE I. CHEMICAL NAMES AND STRUCTURES (Cont.)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

006937

DEC 8 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Atrazine Registration Standard: Mutagenicity Testing Requirement

FROM: Kerry L. Dearfield, Ph.D. 12-7-88
Geneticist
Science Support Section
Science Analysis and Coordination Branch
Health Effects Division (TS-769C)

TO: Marion Copley, D.V.M.
Acting Section Chief
Section 2
Toxicology Branch I - IRS
Health Effects Division (TS-769C)

THRU: John Quest, Ph.D. 12-7-88
Chief
Science Support Section
Science Analysis and Coordination Branch
Health Effects Division (TS-769C)

12/8/88

Atrazine CAS No. 1912-24-9 Tox. Chem. No. 63

I. Background

As of my previous memo to you dated Aug 19, 1988, Ciba-Geigy had not fulfilled the minimum requirements for mutagenicity testing as required by the OPP. The only acceptable tests that had been submitted fulfilled only one of the three mutagenicity testing categories, i.e. gene mutations (e.g. acceptable Salmonella assay). The two other categories had not been fulfilled, i.e. structural chromosome aberrations and other genotoxic effects. In a subsequent submission from Ciba-Geigy, an acceptable mouse micronucleus test (MRID # 407223-01) was reported and minimally fulfilled the structural chromosome aberration category. A recent Ciba-Geigy letter (dated June 29, 1988) mentioned that a UDS assay in rat hepatocytes they performed was earlier considered acceptable; however, upon rereview, it was downgraded to unacceptable for reasons outlined in a memo dated April 26, 1988 from this reviewer to Robert Taylor. Therefore, one mutagenicity category (other genotoxic effects) remains unfulfilled for registration purposes.

The submitted negative mouse micronucleus assay would now minimally fulfill the structural chromosome aberration category. However, it may still not alleviate our concern about atrazine's potential genotoxicity in vivo. A published report (Adler, Mutat. Res. 74: 77-93, 1980) suggested that atrazine induced a positive increase in aberrations at a dose of 2000 mg/kg by oral gavage in olive oil. The submitted micronucleus test, although found negative, tested to a similar level of 2250 mg/kg by oral gavage in CMC. In another Ciba-Geigy submission in which Dr. David Brusick performs an assessment on atrazine (dated December, 1987), Brusick makes several points relevant here: the micronucleus assay would not totally offset the reported positive bone marrow metaphase assay because (a) the metaphase assay is generally considered to be more sensitive, and (b) the negative assays were performed at roughly equivalent dose levels. Also, vehicle effects may influence the results. The positive study used olive oil and the negative study used CMC. Brusick again points out that a closer inspection of olive oil studies would be valuable and that the bioavailability of atrazine from CMC might be investigated. Overall, it would have been useful to repeat the published study to address the possible concern for atrazine mutagenicity. Additional testing for dominant lethal effects, effects by plant metabolites and possibly aneuploidy was also recommended in the April 26, 1988 memo.

II. Recent communication from Adler on published paper

This reviewer has been in contact with the author of the Adler paper published in Mutation Research. Dr. Adler has sent to OPP the summarized Progress Reports of the atrazine work performed by her and her colleagues. These include summary tables of their data for our evaluation. Summarized progress reports of four studies submitted by Dr. Adler included 1) induction of dominant lethals in male mice, 2) spot test for somatic mutations in mice, 3) chromatid aberrations in mouse bone marrow, and 4) micronuclei in polychromatic erythrocytes of mouse bone marrow. The first three studies are reported positive by the investigators and the micronucleus test negative. It should be noted that raw data and complete protocols were not provided and that Dr. Adler plans to publish this information in 1989. While each of these studies individually would not be classified as acceptable to satisfy the different categories for mutagenicity testing (e.g. incomplete protocols; no report of positive controls; use of only one sex in the micronucleus assay), the data provide enough information to elicit a concern for a possible mutagenicity concern for atrazine. It is the responsibility of the Agency to be aware of such concerns and address them. That was the intention of the April 26, 1988 memo from this reviewer to Robert Taylor when it stated that the reported positive published studies should be examined in more detail. The registrant should address these concerns with acceptable submitted studies.

Atrazine was examined for dominant lethal effects in hybrid male (101xC3H)F_1 mice (investigators were U.H. Ehling and J. Kratochvilova). Male mice were exposed to atrazine at a dose of 2000 mg/kg by oral intubation in olive oil. This dose was lethal to 14% of the exposed mice. Males were mated to females for up to 48 days post-treatment in 4 day mating intervals (new females every 4 days). There was a low frequency of fertile matings in the first mating period (38.6% compared to 96% frequency for the control). However, once pregnant, females appeared to have a comparable number of corpora lutea and implants as the controls. There was a slight increase in dominant lethal mutations in the first 3 mating periods (i.e. first 12 days mating post-treatment) as evidenced by an increase in the percent dead implants over controls.

A spot test was performed to examine for presumed somatic mutations in mice (investigator was A. Neuhauser-Klaus). Embryos were treated in utero with atrazine on the ninth day after conception by oral administration to the mothers. Three experiments were reported. The original experiment administered atrazine in olive oil at single doses of 600, 800, 1000 and 1250 mg/kg. Offspring were examined for color spots at 2-3 weeks after birth and then again at 3-4 weeks. Toxicity was seen as an increase in sterile females up to 800 mg/kg. Higher doses killed some females (precise numbers not provided). However, litter sizes at weaning were not influenced. No evidence in an increase of color spots was evident after atrazine administration up to 800 mg/kg in olive oil (no data at higher dosing). Atrazine was orally administered in corn oil with 0.2% tocopherolacetate at 600 mg/kg in the remaining two experiments. Other conditions appeared similar to the original experiment. In one of these experiments, atrazine increased the number of color spots over control in a statistically significant manner ($p=0.031$), whereas in the other experiment, there was an increase, but was not statistically significant ($p=0.061$). The investigator examined the results in all three experiments and found they were homogenous within control and within experimental groups. Therefore, the data were pooled and a statistically significant difference between controls and treated groups was found ($p=0.007$). The author concluded that 600 mg/kg atrazine induced spots when suspended in oil. Only spots of genetic relevance were analyzed (i.e. white mid-ventral spots not included). While these data do not impact on heritable risk, they suggest there may be a concern for somatic mutations and perhaps for reproductive/developmental effects.

Two in vivo cytogenetic studies were performed with atrazine (investigators were U. Kliesch and I.-D. Adler). Abarrations and micronuclei were assayed in bone marrow from hybrid (101xC3H)F_1 male mice after oral administration of atrazine. The aberration study is the one mentioned above (Part I). A single oral dose of

atrazine at 2000 mg/kg in olive oil was given to 8 animals. Bone marrow was obtained 24 hours after treatment and 125 mitoses/animal scored. An average aberration frequency increase of 4.1% vs. 0.7% for controls was found. Only deletions of the chromatid type were reported. In the micronucleus experiment, a single oral dose of atrazine at 100, 500 or 1000 mg/kg in DMSO was given to 4 animals/dose/sacrifice time. Bone marrow was obtained at 24, 48, 72, 96 and 120 hours after treatment and 2000 polychromatic erythrocytes/animal were scored. Negative results were obtained. In this same report, an additional data entry appears to show that atrazine was also tested in the micronucleus test at a single dose of 2000 mg/kg in olive oil and bone marrow obtained 24 hours after treatment. These results were also negative. The negative micronucleus results appear consistent with the registrant's own micronucleus test results performed in a different mouse strain (MRID #407223-01).

III. Requirements for Registration Standard

As far as minimal requirements for mutagenicity testing is concerned, the category for other genotoxic effects has not been fulfilled. This is a data gap that should be addressed in the Registration Standard. Tests that may be appropriate here may examine for aneuploidy and/or the impact of plant metabolism. Since these are not routine tests, the selection of what test(s) to perform for this category should be discussed with the OPP.

The results from the dominant lethal assay suggest that there may be a concern for heritable risk from atrazine exposure. The reduced fertility frequency indicates that there is exposure to the germ cells in the exposed males. The slight increase in dominant lethal effects suggests that there may be genetic alterations that could be transmissible. It should be noted that since the results are not overwhelmingly positive, the results alone do not suggest a very high priority concern for heritable risk. However, atrazine warrants further examination due to the potential exposure to humans. Atrazine is among the most commonly occurring pesticides in ground water. It is also found in surface waters. The high frequency of atrazine detection in ground water is related to its high volume of use. More pounds of atrazine active ingredient are applied in the United States annually than any other pesticide (with the possible exception of alachlor). In addition, for applicator exposures, there are many instances where the margins of safety and margins of exposure are considered to be of toxicological concern. With this high potential for human exposure, the Agency should deal with any concern for potential health effects, heritable risks included.

Ciba-Geigy has also performed a dominant lethal test in mice (Document #005833). However, this assay was considered unacceptable despite the collection of additional information concerning this dominant lethal assay. It should be noted that

this endpoint was a concern as Adler (1980) reported a positive effect in this assay (discussed above). The Agency would like to see this result addressed. Brusick states in his review that the positive effects were found at a higher dose level than that of the submitted dominant lethal study and since there were no other confounding factors, the positive results are accepted in his analysis. Again, it should be mentioned that vehicle effects may play a role here (the positive study used olive oil, the negative study used CMC).

It is suggested for the Registration Standard that an acceptable dominant lethal assay with male mice be performed with atrazine active ingredient. Since it is the intention to reproduce the published study's results, it is highly recommended that the registrant discuss with the OPP the protocol(s) for which to perform this test (e.g. selection of doses, dose range, vehicle, animal strains). This test should be able to be completed and reported to the OPP within a one year time period.

006937

Reviewed by: Sanford W. Bigelow, Ph.D. 5/6/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W Hauswirth 5/9/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 63

ACCESSION NUMBER: MRID NO.: 404375-01

TEST MATERIAL: Atrazine

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

STUDY NUMBER: ABR-87116

SPONSOR: CIBA-GEIGY Corp., Agricultural Division, P.O. Box 18300 Greensboro, NC 27419 Thomas Parshley, Regulatory Specialist (919) 292-7100 X7207

TESTING FACILITY: CIBA-GEIGY Corp., Biochemistry Dept., P.O. Box 18300 Greensboro, NC 27419

TITLE OF REPORT: A Summary of the Disposition, Kinetics and Metabolism of Atrazine in the Rat (General Metabolism).

AUTHOR: G.R. Orr

REPORT ISSUED: November 17, 1987

CONCLUSIONS:

The summary data regarding the distribution, metabolism and the elimination of atrazine were provided in this report. To this end, three separate experiments were conducted with the use of three groups of rats. Radiolabeled atrazine (triazine ring, uniformly labeled) was used by the author to measure the disposition of atrazine and/or its metabolites in the rat. The first experiment was performed to assess the distribution and elimination of atrazine in male and female rats repeatedly exposed to daily doses of atrazine. The second experiment was performed to assess in further detail the distribution of atrazine in female rats, especially in the red blood cell. The third experiment was conducted to identify the urinary metabolites of atrazine formed by the female rat. The absorption of atrazine in male or female rats was not reported.

Excretion of atrazine in rats. About 95% of the atrazine administered orally is excreted within 7 days after cessation of exposure. The route of atrazine excretion was reported to be independent of the dose and sex of rat. About 75% of the atrazine is excreted through the urinary route whereas about 20% of the atrazine is eliminated in the feces. The elimination route for the remaining 5% was not reported. Also, the level of atrazine elimination by exhalation or through the skin (sweating) was not reported.

Metabolism of atrazine in rats. The data reported in this study indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alkyl substituents of atrazine appears to be a minor and secondary metabolic route.

The author argues that a "carbon-sulfur lyase," cleaves the glutathione residue from an atrazine metabolite to produce a thiol-containing atrazine metabolite. The author further posits that the action of the lyase results in the covalent binding of the thiol-containing atrazine metabolite to hemoglobin in the red blood cell, a finding from the multiple exposure studies (depicted in Table 7). However, the author has not provided evidence in this study whether lyase is present in red blood cells.

Summary. The whole body half-life of 1.61 days for atrazine is consistent with the observation that 95% of the administered dose is elimination within 7 days after exposure. The rad cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat.

Classification: **Acceptable:** This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied only for reporting (1) the identity of urinary metabolites of atrazine in female rats as well as (2) the distribution and excretion of atrazine in male and female rats. However, all of the data requirements for metabolism studies set forth in Subdivision F §85-1 have not been reported, i.e., (a) the urinary and fecal metabolites of atrazine in male rats and (b) the fecal metabolites of atrazine in females must be identified to completely satisfy the §85-1 data reporting requirements for the metabolism of atrazine in the rat.

Table 2
Animal Assignment in this Study
(Atrazine Distribution Experiment)

Test Group	Daily Oral Dose Given (mg/kg)	Rats (female)	Duration of Exposure (days)
1 Control	0	2	10
2 Low1 (LDT1)	1.0	2	10
3 Low2 (LDT2)	3.0	2	10
4 Low3 (LDT3)	7.0	2	10
5 Low4 (LDT4)	10.0	2	10
6 Mid1 (MDT1)	50.0	2	10
7 High (HDT1)	100.0	2	10

Table 3
Animal Assignment in this Study
(Atrazine Metabolism Experiment)

Test Group	Daily Oral Dose Given (mg/kg)	Rats (female)	Duration of Exposure (day)
1 High	100.0	5	1
2 Mid	16.2 - 19.6	8	1

B. Diet Preparation: Atrazine was was given orally to the rats (via a stomach tube) as an active ingredient or as a radiolabeled active ingredient. Animals were allowed free access to animal feed (Purina) and tap water. The animals were allowed a one-week acclimation period prior to initiation of experimentation.

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this summary report.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

B. Atrazine dosage regimens: Three separate experiments were conducted with the use of three groups of rats. The first experiment was performed to assess the distribution and elimination of atrazine. The second experiment was performed to assess in further detail the distribution of atrazine, especially in the red blood cell. The third experiment was conducted to identify the atrazine metabolites formed by the rat.

1. Experiment #1. As shown in Tables 1, 2, and 3, respectively, three groups of rats (5 males and 5 females) were treated orally with atrazine. The first group received a single oral dose of ^{14}C-atrazine at 1 mg/kg; a second group were given a single oral dose of 100 mg/kg ^{14}C-atrazine; and a third group received daily oral doses of 1 mg/kg of nonradiolabeled atrazine for 14 days and on day 15, was given 1 mg/kg ^{14}C-atrazine.

Following the last dose of ^{14}C-atrazine in each group, the feces and urine were collected in each animal for 7 days. Following this, the rats were sacrificed and the urine, feces, and red blood cells, and the following selected tissues were analyzed for ^{14}C content (Figure 1).

in each group was sacrificed 3 hours after the tenth dose of ^{14}C-atrazine and the other animal in each group was sacrificed 72 hours after the tenth dose of ^{14}C-atrazine. The distribution of ^{14}C-label in the urine, feces, red blood cells, and the following selected tissues was determined for each female rat (Figure 2).

FIGURE 2

Digestive system		**Cardiovascular**		**Neurological**			
	Tongue		Aorta*		X	Brain*+@	
	Salivary glands*		Heart*@			Peripheral nerve*#	
	Esophagus*		Bone marrow*#			Spinal cord (3 levels)*#	
	Stomach*		Lymph nodes*		X	Pituitary*	
	Duodenum*		Spleen@			Eyes (optic n.)*#	
	Jejunum*		Thymus*				
	Ileum*	X	Red blood cell			**Glandular**	
	Cecum*	**Urogenital**				Adrenal gland*	
	Colon*	X	Kidneys*+@			Exorbital lacrimal gland#	
	Rectum*		Bladder*		X	Mammary gland*#	
X	Liver *+@		Testes*+@			Parathyroids*++	
	Gall bladder*#		Epididymides			Thyroids*++	
	Pancreas*		Prostate		**Other tissues**		
Respiratory			Seminal vesicle			Bone (femur)*#	
	Trachea*#	X	Ovaries*+@			Muscle*#@	
	Lung*@		Uterus*@			Skin*#	
	Nose^		Cervix			All gross lesions and masses*	
	Pharynx^		Fallopian tubes			Residual Carcass@	
	Larynx^					Fat@	
						Plasma (blood)@	

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
@ Required for determining distribution in metabolism studies.

V. RESULTS:

A. Distribution and Elimination of Atrazine and Its Metabolites

Experiment #1. The 5 male and 5 female rats were used to assess the disposition and elimination of atrazine after single or multiple oral doses of atrazine. Table 4 shows that the total recovery of atrazine averaged 102.9% for the group given a single dose of 1 mg/kg ^{14}C-atrazine, 103.2% for the group of rats given a single dose of 100 mg/kg ^{14}C-atrazine, and 88.3% for the group of rats given a daily dose of 1 mg/kg atrazine followed by a single dose of 1 mg/kg ^{14}C-atrazine on day 15 (referred here as the multiple dosing or the multiple exposure group).

Concerning the elimination of atrazine or its metabolites, approximately 95% of the ^{14}C-label was excreted within 7 days of the last exposure (Table 4). In all 3 groups of rats, roughly 75% of the ^{14}C-label was excreted in the urine whereas about 20% of the ^{14}C-label was eliminated in the feces. Both discussion of other routes of elimination and the remaining 5% of the administered atrazine were not reported.

However, differences between dosage groups for tissue-borne ^{14}C-label were observed. A statistically significant decrease ($p < 0.05$) in the mean level of tissue-borne ^{14}C-label was found in those rats given a single dose of 100 mg/kg when compared to the group of rats who received a single dose of 1 mg/kg atrazine. Also, a statistically significant decrease ($p < 0.05$) in the mean level of tissue-borne ^{14}C-label was found in those rats treated with multiple oral doses of atrazine when compared to the group of rats who received a single oral dose of 1 mg/kg ^{14}C-atrazine. No differences were observed between sexes regarding the percentage of ^{14}C-label that was excreted in the urine and feces (Table 4). The pattern for tissue distributed between single and multiple exposure groups were similar (Table 5) collected 7 days after exposure to ^{14}C-atrazine.

The red blood cells (RBC) had the highest levels of ^{14}C-label of all tissues studied (Table 5). The ratio of RBC binding of the ^{14}C-label was proportional to the dose administered, i.e., the concentration for the high dose single exposure group (100 mg/kg) was about 100 times that of the low dose single exposure group (1 mg/kg), and the tissue concentration of the multiple dose group (1 mg/kg for 15 days) was the same (1.11 and 1.00) to that of the

Table 4

Distribution and Elimination of ^{14}C-Label (ppm) After 7 Days Following ^{14}C-Atrazine Treatment (taken from Table I)

Dose	1.0 mg/kg		100.0 mg/kg		1.0 mg/kg (multiple doses)	
Sex (#)	Males(5)	Females(5)	Males(5)	Females(5)	Males(5)	Females(5)
Urine	0.77 ±0.01	0.77 ±0.02	77.27 ±1.67	79.86 ±2.16	0.67 ±0.04	0.62 ±0.09
Feces	0.18 ±0.01	0.19 ±0.01	21.34 ±0.55	17.85 ±0.71	0.19 ±0.01	0.17 ±0.01
Tissues	0.06 ±0.001	0.07 ±0.001	4.98 ±0.13	4.48 ±0.34	0.047 ±.002	0.046 ±0.002
Cage wash	0.002 ±0.0004	0.003 ±0.001	0.33 ±0.08	0.29 ±0.11	0.005 ±0.001	0.006 ±0.001
Total	1.02 ±0.01	1.04 ±0.01	103.92 ±1.44	102.48 ±2.89	0.92 ±0.44	0.85 ±0.09
% Recovery	102.9 ±1.1		103.2 ±1.5		88.3 ±4.9	

B. Distribution of radiolabeled atrazine after repeated daily dosing and multiple sampling.

Another experiment was conducted with a protocol designed to determine the bodily disposition of ^{14}C-label after multiple doses of ^{14}C-atrazine. The recovery of the total dose averaged 89.2% in rats killed 3 hours after the tenth dose of ^{14}C-atrazine and 94.2% in rats killed 72 hours after the tenth dose of ^{14}C-atrazine averaged. The amount of ^{14}C-label of the total dose excreted in the feces in rats killed at 3 hours was 13.4% and was 14.8% in rats killed at 72 hours independent of the dose. The amount of ^{14}C-label of the total dose excreted in the urine was 69.5% in the rats killed at 3 hours and 76.3% in the rats killed at 72 hours independent of the dose. The total percentage of the initial dose ^{14}C-atrazine excreted in the urine and feces in the rats killed at 3 hours was 82.9% and in the rats killed at 72 hours was 91.1%.

Plasma concentrations of atrazine. In this multiple dosing experiment, plasma concentrations were related linearly to the dose of ^{14}C-atrazine (Table 6). That is, plasma concentrations in rats given 100 mg/kg ^{14}C-atrazine were roughly 100 times that of rats given 1 mg/kg ^{14}C-atrazine. This comparison applies to all of the dosage groups at most time points listed in Table 6. Overall, during daily dosing plasma levels of atrazine or its metabolites generally rose and reached an apparent plateau or steady-state. After daily dosing had stopped the following toxicokinetic values were calculated from the data obtained:

- the whole body half-life, or $t_{1/2}$, of 38.6 hours (1.61 days) for the elimination of atrazine or its metabolites,
- the estimated volume of distribution, or V_d, for the daily dose of 10 mg/kg was 4.15 L/kg, and
- at a dose of 10 mg/kg, the mean plasma concentration of atrazine or its metabolites at steady-state was 5.61 mg-equivalents ^{14}C-label/L of plasma.

For distribution models that follow first-order kinetics such as this model proposed for atrazine, two relationships are found: (1) $t_{1/2}$ and V_d are independent of the dose and (2) the plasma concentration of ^{14}C-label is directly proportional to the dose of ^{14}C-atrazine.

RBC concentrations of atrazine. The same experimental method used for determining plasma concentrations of atrazine and its metabolites was employed to measure the level of ^{14}C-label in red blood cells (RBCs). The concentration of ^{14}C-label in RBCs rose during repeated daily dosing of ^{14}C-atrazine and did not reach a plateau or steady state (Table 7). RBC concentrations appeared to be proportional (usually supralinear) to the dose of ^{14}C-atrazine. After cessation of daily dosing, the concentration of ^{14}C-label declined for all doses except the highest dose, 100 mg/kg ^{14}C-atrazine.

After daily dosing was stopped, the data was obtained from the level of ^{14}C-label in the urine. The following toxicokinetic values were calculated from those data:

- the mean dosage half-life, or $t_{1/2}$, was 1562.9 hours (8.14 days) for the elimination of atrazine or its metabolites from RBCs,
- the estimated volume of distribution, or V_d, for the daily dose of 10 mg/kg was 0.7 L/kg, and
- at a dose of 10 mg/kg, the mean plasma concentration of atrazine or its metabolites at steady-state was 104.6 mg-equivalents ^{14}C-label/L of cells.

The RBC:plasma concentration ratio was roughly related linearly in all dose levels. The estimated half-life of 8.14 days and the large volume of distribution 104.6 mg-equivalents/L) in RBCs indicate that extensive binding of atrazine and its metabolites in RBCs were occurring. (The life span of a rat RBC is 45-56 days). The authors speculate that binding of ^{14}C-label is of a covalent nature.

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Tissue concentrations of atrazine. The tissue concentrations of atrazine and its metabolites were measured in selected tissues from animals killed at 3 and at 72 hours (Table 8). At all doses, tissue levels of ^{14}C-label are consistently lower in all animals killed 72 hours after cessation of ^{14}C-atrazine exposure, a finding that corroborates the observed decline in plasma concentration of ^{14}C-label (Table 6). The liver had the highest tissue concentration of ^{14}C-label, followed by the kidney, pituitary and ovary. The brain had the lowest tissue concentration in this experiment. In respect to making dose comparisons, tissue levels of ^{14}C-label were generally supralinear, i.e., the tissue level in rats given 100 mg/kg ^{14}C-atrazine was generally 200 times higher than that of rats given 1 mg/kg ^{14}C-atrazine. In animals sacrificed at 72 hours, the mammary tissue:plasma concentration ratio at 1 mg/kg was 0.042 and at 100 mg/kg was 0.49; a difference that is roughly proportional to the dose of atrazine.

B. The Metabolism of Atrazine

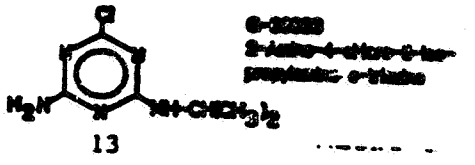
To examine the metabolism of atrazine in rats, 100 mg/kg of ^{14}C-atrazine was given to rats and the ^{14}C-labeled metabolites were isolated and identified. A recovery of 103.78% of the total radioactivity was achieved. The urinary route accounted for 47.4% of the elimination whereas 49.3% of the ^{14}C-label was eliminated via the fecal route. The tissues contained 5.75% of the ^{14}C-label while the blood contained the remaining 1.4% of the ^{14}C-label.

In vivo metabolism of atrazine. The molecular structures of the urinary metabolites obtained from the first group rats were unattainable, so a second group of 8 rats were given 16.18-19.64 mg/kg ^{14}C-atrazine. The metabolites were collected within the 0 to 24 hour time period after exposure. The urine was freeze dried. Then the metabolites were dissolved in a small amount of water that was acidified with HCl to pH 3.0 and separated with an amino acid analyzer (to detect the amino acid residues of glutathione) coupled with a cation exchange column.

A total of 19 radioactive peaks were detected, three of which were identified as metabolites by comparison of the infrared and mass spectra. The identity of two other metabolites was postulated based on additional mass spectral information. The molecular structures of some of the atrazine metabolites are shown in Figure 1 and the numbers in this figure correspond to the metabolites discussed in the text. Eight metabolites were identified and the major metabolites are listed below:

- 2-hydroxy-atrazine (7),
- 2-hydroxy-4-amino-6-isopropylamino-s-triazine (8),
- 2-hydroxy-4-ethylamino-6-amino-s-triazine (14), and
- 2-hydroxy-4,6-diamino-s-triazine (3).

The identification of the major metabolites above indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Because four other minor metabolites that possess omega-carboxyl moieties were identified (5, 10, 11, 12), oxidation of the terminal methyl moieties in the alkyl substituents appears to be a minor and secondary metabolic route.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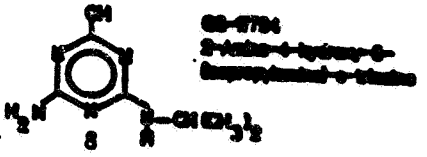
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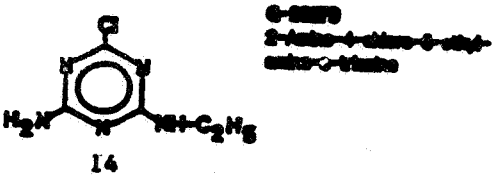
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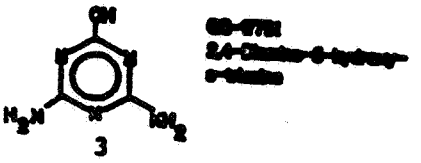
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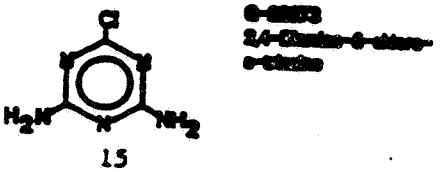
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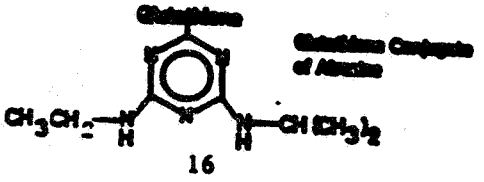
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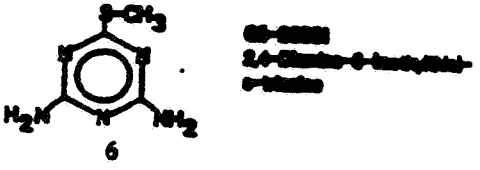
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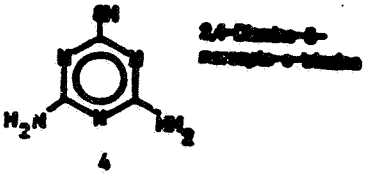
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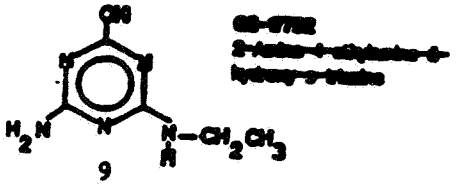
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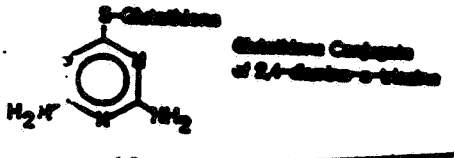
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FIGURE I. CHEMICAL NAMES AND STRUCTURES (Cont.)

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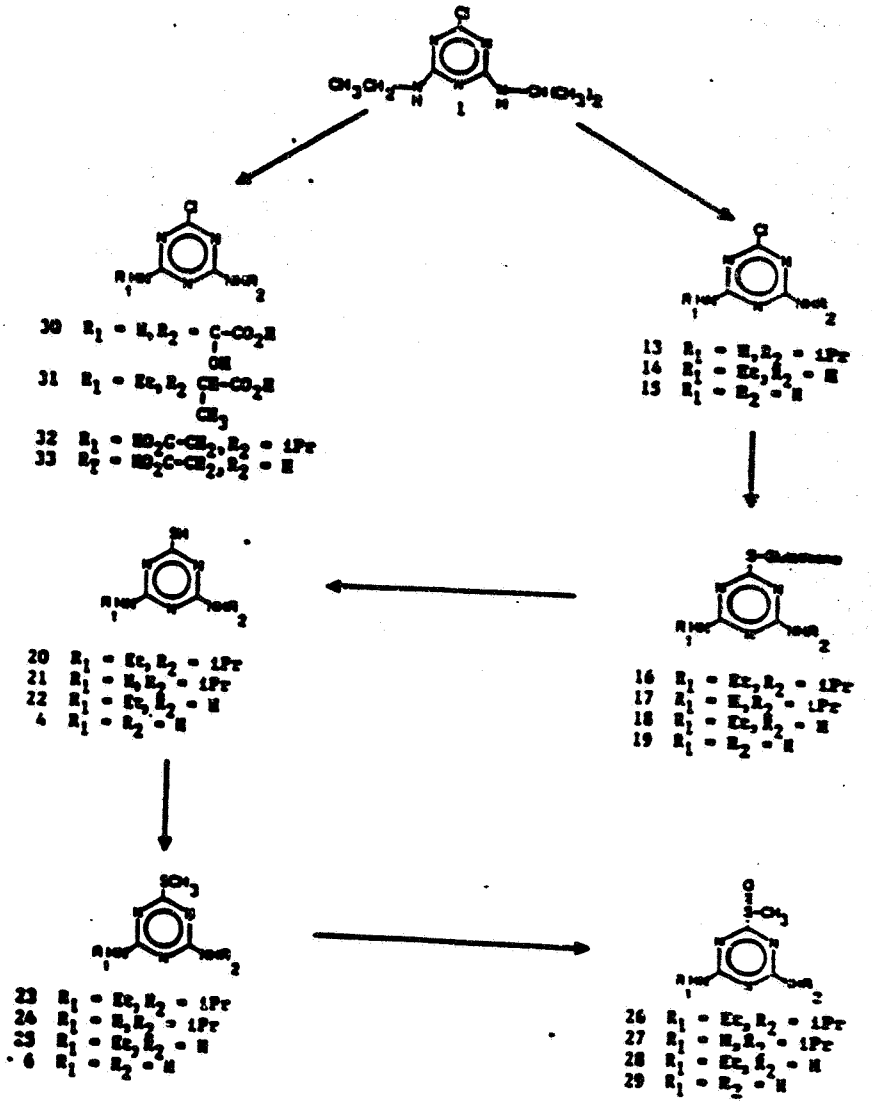

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FIGURE 2. PROPOSED METABOLIC PATHWAY FOR ATRAZINE IN THE RAT

proportional to the dose of atrazine. Plasma concentrations of atrazine measured during and after atrazine exposure showed that the whole body half-life ($t_{1/2}$) of atrazine or its metabolites is 38.6 hours (1.61 days) in rats. These reported findings further indicate that atrazine does not accumulate under this exposure regimen in the rat.

As mentioned above, the highest level of atrazine was found in the RBC. The estimated half-life of 8.14 days in RBCs (as compared to the whole body half-life of 1.61 days) indicates that extensive binding of atrazine or its metabolites in RBCs was occurring. However, after cessation of multiple exposure, the concentration of atrazine or its metabolites in RBCs declined at all doses except for the highest dose, 100 mg/kg atrazine.

Excretion of atrazine in rats. About 95% of the atrazine administered orally is excreted within 7 days after cessation of exposure. The route of atrazine excretion was reported to be independent of the dose and sex of rat. About 75% of the atrazine is excreted through the urinary route whereas about 20% of the atrazine is eliminated in the feces. The elimination route for the remaining 5% was not reported. Also, the level of atrazine elimination by exhalation or through the skin (sweating) was not reported.

Metabolism of atrazine in rats. The data reported in this study indicates that dechlorination of the triazine ring and N-dealkylation are the major metabolic pathways for atrazine in rats. Oxidation of the alkyl substituents of atrazine appears to be a minor and secondary metabolic route.

The author argues that a "carbon-sulfur lyase," cleaves the glutathione residue from an atrazine metabolite to produce a thiol-containing atrazine metabolite. The author further posits that the action of the lyase results in the covalent binding of the thiol-containing atrazine metabolite to hemoglobin in the red blood cell, a finding from the multiple exposure studies (depicted in Table 7). However, the author has not provided evidence in this study whether lyase is present in red blood cells.

Summary. The whole body half-life of 1.61 days for atrazine is consistent with the observation that 95% of the administered dose is elimination within 7 days after exposure. The red cells store the highest concentration of atrazine in the rat, apparently through the covalent binding of a metabolite. Under the dose regimen employed in this study, atrazine does not accumulate in the rat.

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Data Evaluation Report

006718

Compound Atrazine

Citation

Dermal absorption of ^{14}C-Atrazine by rats (general metabolism), T. Murphy, Biochemistry Dept., Agricultural Division, Ciba-Geigy Corp. Study No. ABR-87098; 11/6/87, MIRD 404313-08.

This document contains the following report which describes the in life portion of the study;

Dermal absorption of ^{14}C-Atrazine in Rats, E.M. Craine, WIL Research Laboratories, Project No. WIL-82015, 11/5/87.

Reviewed by Robert P. Zendzian Ph.D. 3/24/88
Senior Pharmacologist

Core Classification Acceptable

Conclusions

Atrazine in 4L formulation is absorbed in relatively small amounts through the skin. Typical values are 2.00, 0.53 and 0.26 % for 10 hour exposures to doses of 0.01, 0.1 or 1.0 mg/cm^2. Significant quantities remain on the skin after washing with soap and water (24.87, 21.10 and 10.49 %). No significant differences in absorption were observed between the 4L and 80W formulations tested at 1.0 mg/cm^2 for 10 hours. The data indicate that absorption is approaching saturation at the high dose.

Materials

Artazine uniformly ring labeled,

low and mid doses
22.0 uCi/mg, 99.5%

high doses
2.3 uCi/mg, 99.0%

Crl:CD®BR male rats 27-41 days old from Charles River Breeding laboratories

Experimental design and methods

Dose preparation and sample analysis was performed at Ciba-Geigy and the in life portion of the study at WIL.

"The low dose was prepared by mixing throughly 4.0 mg of ^{14}C-Atrazine and 5.3 mg of the formulant (4L), then suspending the mixture in 2.0 ml of deionized water. The middose was

prepared by mixing 40 mg of ^{14}C-Atrazine and 53.0 mg of blank formulation (4L) and then suspending the mixture in 2.0 ml of deionized water."

"The 4L high dose formulation was prepared by mixing throughly 530 mg of formulant and 400.0 mg of ^{14}C-Atrazine, then suspending the mixture in 4.0 ml of water. The 80W high dose was prepared by mixing 200.0 mg of ^{14}C-Atrazine and 50.0 mg blank formulant, then suspending the mixture in 2.0 ml of deionized water.

Two groups of 16 and one group of 20 male rats were treated dermally with single doses of ^{14}C-atrazine at 0.1, 1.0 and 10.0 mg/rat (0.01, 0.1 and 1.0 mg/cm^2) respectively. Four animals at each dose were dosed with 4L formulation and exposed for 2, 4, 10 and 24 hours. The remaining four animals at 10.0 mg/rat were dosed with 80W formulation and exposed for 10 hours.

"The test material preparations were stored frozen, warmed to room temperature and sonicated 10 minutes prior to analysis and dosing on the appropriate test material application day."

The anterior dorsal hair was shaved from each rat and the area washed with acetone 24 hours prior to dosing. Test material was applied to a 2.5 x 4 cm (10cm2) area by pipette. The application site was covered with a protective device consisting of a stomahesive bandage as a wall and a filter paper cover.

Animals were individually caged in metabolism cages and total urine and feces collected.

Animals were sacrificed at the end of the exposure period. The protective device was removed and washed. The application site was washed with a detergent solution and water rinsed.

Blood, application site skin, skin under the bandage and the carcass were collected.

The following samples from each animal were sent to Ciba-Geigy for analysis;

"pipet washes, urine, feces, washes, extracts, samples from the protective coverings, gauze, blood, skin samples and carcasses,"

Results

Sample analysis for radioactivity at WIL indicated that dosing suspensions were homogenous and of the expected activity.

No compound-related effects on the rats were reported.

Dermal absorption data is summarized in Table 1 below and presented in detail in Tables III - VI of the report.

Table 1. Summary of dermal absorption data. All values are means of 4 animals. All animals dosed with 4L formulation except as noted. Data from Tables III - VI of the report.

Dose (mg/cm^2)	Exposure (hours)	Absorbed[a] (%)	(%/hr)	(mgx10-5)	On skin[b] (%)	Unabsorbed[c] (%)
0.01*	2	0.68	0.34	6	23.53	77.25
0.009†	4	1.24	0.31	11	20.56	71.88
	10	2.00	0.20	18	24.87	69.51
	24	4.93	0.21	44	20.72	69.02
0.1	2	0.21	0.11	20	25.06	71.55
0.095	4	0.36	0.09	34	18.97	75.72
	10	0.53	0.05	50	21.10	78.93
	24	1.26	0.05	119	29.04	67.43
1.0	2	0.13	0.06	107	11.24	88.67
0.82	4	0.09	0.02	74	14.69	88.00
	10	0.26	0.03	213	10.49	89.29
	24	0.21	0.01	172	9.58	91.03
1.0 80W	10	0.24	0.02	244	8.81	89.15
1.02						

* Nominal dose.
† Applied dose.
a. Total of blood, carcass, urine and feces.
b. Total of skin I and skin II.
c. Total of bandage rinse, bridge rinse, paper rinse, soap rinse, water rinse, gauze A, gauze B and cage wash.

Discussion

The percent of dose absorbed followed the most common pattern of absorption with the percent increasing with time and decreasing with increasing dose. Significant quantities of test material remained on/in the skin following soap and water wash. There are clear indications that the process is approaching saturation at the high dose in that;

1. The percent absorbed per hour decreased with time in each dose and the proportionate decrease was larger with increasing dose.

2. As the dose increased the total quantities absorbed increased proportionately less per dose increase.

3. The quantity on/in the skin increased ten fold from 0.01 to 0.1 mg/cm^2 but only five fold from 0.1 to 1.0 mg/cm^2.

For regulatory purposes the test material which remains on/in the skin after soap and water wash is considered absorbable. For risk assesments the percent absorbed is added to the percent on/in the skin to determining quantity absorbed. However, the possibility exists that the relatively large quantity remaining on/in the skin is an artifact of the experimental procedure. A recent study, designed to determine if the material remaining on/in the skin after washing could be absorbed, showed that 2 to 3 times more material could be washed from the skin of living animals then from the skin of recently sacrificed animals. In this study the animals were sacrificied before washing the application site.

This possibility may be tested by treating 4 animals per dose for 10 hours exactly as was done in this study but washing the appliction site before sacrificing the animals. The ten hour exposure time is suggested as modeling a worker who washes at the end of the working day.

ATRAZINE 080803

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Pages 337 through 340 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

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____ Sales or other commercial/financial information.

____ A draft product label.

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____ Information about a pending registration action.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

C. Statistics:

The following procedures were utilized in analyzing the numerical data:

One- and two-way analysis of variance (ANOVA) was used to assess the statistical significance of results between dose, treatment groups or sex. When appropriate, Dunnetts or Newman-Keuls t-tests were performed to assess differences between group means.

For generating the kinetic models, the excretion data was used. This evaluation was performed by I.W.F. Davidson of Bowman Gray School of Medicine (Wake Forest University). The evaluation was limited because of the low number of rats used in each group. Additional kinetic parameters such as rate constants, half-life values, and alpha and beta distribution values were obtained with the use of the ESTRIP and PCNONLIN computer programs calculated by C.M. Metzler and D.L. Weiner (Statistical Consultants, Edgewood, KY).

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector from (1) SRI International, the subcontracting laboratory where the distribution of radiolabeled atrazine was studied and (2) Agrisearch Incorporated, another subcontracting laboratory where the amount of radiolabeled atrazine was measured.

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this summary report.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study. Rat # 5065 (given 3 mg/kg atrazine for 3 days) favored its right side, and upon examination, the lungs were found to be "present in the lower thoracic area."

B. Experimental Protocol: This experiment was performed to assess in further detail the dose-dependent distribution of atrazine, especially in the red blood cell. As listed in Table 1, in an effort to study in more detail the toxicokinetic disposition of ^{14}C-atrazine as a function of the dose of atrazine and the time of sacrifice, six groups of female Sprague-Dawley rats were treated with ^{14}C-atrazine while another group of female rats served as a control group. The groups of rats were dosed daily for 10 consecutive days at 0 mg/kg (vehicle only), 1 mg/kg, 3 mg/kg, 7 mg/kg, 10 mg/kg, 50 mg/kg, and 100 mg/kg ^{14}C-atrazine. The vehicle was an aqueous solution of corn starch/polysorbate-80.

Urine and feces were collected daily. At 24, 48, 72, 96, 144, 192, 219, 240, 264 and 288 hours, blood samples were obtained via orbital puncture. Five milliliters of blood were collected by aortal puncture at sacrifice. The tissues selected for determining the distribution of ^{14}C-label at each dose are listed in Figure 1. One of the two animals in each group was sacrificed 3 hours after the tenth dose of ^{14}C-atrazine and the other animal in each group was sacrificed 72 hours after the tenth dose of ^{14}C-atrazine. The distribution of ^{14}C-label in the urine, feces, red blood cells, and the following selected tissues was determined for each female rat (Figure 1).

FIGURE 1

	Digestive system		Cardiovascular		Neurological
	Tongue		Aorta*	X	Brain*+@
	Salivary glands*		Heart*@		Peripheral nerve*#
	Esophagus*		Bone marrow*#		Spinal cord (3 levels)*#
	Stomach*		Lymph nodes*	X	Pituitary*
	Duodenum*		Spleen@		Eyes (optic n.)*#
	Jejunum*		Thymus*		
	Ileum*	X	Red blood cell		Glandular
	Cecum*		Urogenital		Adrenal gland*
	Colon*	X	Kidneys*+@		Exorbital lacrimal gland#
	Rectum*		Bladder*	X	Mammary gland*#
X	Liver *+@		Testes*+@		Parathyroids*++
	Gall bladder*#		Epididymides		Thyroids*++
	Pancreas*		Prostate		Other tissues
	Respiratory		Seminal vesicle		Bone (femur)*#
	Trachea*#	X	Ovaries*+@		Muscle*#@
	Lung*@		Uterus*@		Skin*#
	Nose^		Cervix		All gross lesions and masses*
	Pharynx^		Fallopian tubes		Residual Carcass@
	Larynx^				Fat@
					Plasma (blood)@

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
@ Required for determining distribution in metabolism studies.

V. RESULTS:

A. Distribution of radiolabeled atrazine after repeated daily dosing and multiple sampling.

The experiment was conducted with a protocol designed to determine the bodily disposition of ^{14}C-label after exposure for 10 days to a number of doses of ^{14}C-atrazine. The recovery of the total dose averaged 89.2% in rats killed 3 hours after the tenth dose of ^{14}C-atrazine and 94.2% in rats killed 72 hours after the tenth dose of ^{14}C-atrazine averaged. The amount of ^{14}C-label of the total dose excreted in the feces in rats killed at 3 hours was 13.4% and was 14.8% in rats killed at 72 hours independent of the dose. The amount of ^{14}C-label of the total dose excreted in the urine was 69.5% in the rats killed at 3 hours and 76.3% in the rats killed at 72 hours independent of the dose. The total percentage of the initial dose ^{14}C-atrazine excreted in the urine and feces in the rats killed at 3 hours was 82.9% and in the rats killed at 72 hours was 91.1%.

Plasma concentrations of atrazine. In this experiment, plasma concentrations were related linearly to the dose of ^{14}C-atrazine (Table 2). That is, plasma concentrations in rats given 100 mg/kg ^{14}C-atrazine were roughly 100 times that of rats given 1 mg/kg ^{14}C-atrazine. This comparison applies to all of the dosage groups at most time points listed in Table 2. Overall, during daily dosing plasma levels of atrazine or its metabolites generally rose and reached an apparent plateau or steady-state. After daily dosing had stopped the following toxicokinetic values were calculated from the data obtained:

- the whole body half-life, or $t_{1/2}$, of 38.6 hours (1.61 days) for the elimination of atrazine or its metabolites,
- the estimated volume of distribution, or V_d, for the daily dose of 10 mg/kg was 4.15 L/kg, and
- at a dose of 10 mg/kg, the mean plasma concentration of atrazine or its metabolites at steady-state was 5.61 mg-equivalents ^{14}C-label/L of plasma.

For distribution models that follow first-order kinetics such as this model proposed for atrazine, two relationships are reported: (1) $t_{1/2}$ and V_d are independent of the dose and (2) the plasma concentration of ^{14}C-label is directly proportional to the dose of ^{14}C-atrazine.

Table 2

Plasma Levels of ^{14}C-Label (ppm) During the Dosing Period and at Sacrifice
(taken from Table VIII)

Dose	1 mg/kg		3 mg/kg		7 mg/kg		10 mg/kg		50 mg/kg		100 mg/kg	
Rat #:	R5062	R5063	R5064	R5065	R5066	R5067	R5068	R5069	R5070	R5071	R5072	R5073
Hour of Sacrifice:	3	72	72	3	3	72	3	72	3	72	3	72
Time (hrs.):												
4	0.068	0.061	0.063	0.375	0.741	0.562	1.062	1.164	9.291	8.279	27.104	22.298
8	0.186	0.181	0.452	0.615	1.058	1.884	2.009	1.593	7.161	.1297	28.946	23.101
2	0.469	0.405	1.383	1.468	3.267	3.248	4.168	3.957	20.911	17.778	40.320	57.877
6	0.506	0.596	1.808	1.845	3.311	3.747	4.165	4.661	21.249	24.448	52.271	56.580
44	0.582	0.594	2.150	2.608	4.225	3.359	5.069	5.109	25.169	24.604	59.751	69.514
92	0.560	0.658	1.668	1.941	4.066	3.533	5.343	4.725	23.437	27.682	48.671	44.420
19	0.583	--	--	1.406	3.748	--	5.067	--	21.351	--	51.715	--
40	--	0.185	0.703	--	--	1.628	--	3.099	--	26.413	--	29.566
64	--	0.144	0.789	--	--	1.371	--	1.713	--	13.352	--	17.682
88	--	0.117	0.340	--	--	0.868	--	1.600	--	8.302	--	14.775

RBC concentrations of atrazine. The same experimental method used for determining plasma concentrations of atrazine and its metabolites was employed to measure the level of ^{14}C-label in red blood cells (RBCs). The concentration of ^{14}C-label in RBCs rose during repeated daily dosing of ^{14}C-atrazine and did not reach a plateau or steady state (Table 3). RBC concentrations appeared to be proportional (usually superlinear) to the dose of ^{14}C-atrazine. After cessation of daily dosing, the concentration of ^{14}C-label declined for all doses except the highest dose, 100 mg/kg ^{14}C-atrazine.

After daily dosing was stopped, the data was obtained from the level of ^{14}C-label in the urine. The following toxicokinetic values were calculated from those data:

- the mean dosage half-life, or $t_{1/2}$, was 1562.9 hours (8.14 days) for the elimination of atrazine or its metabolites from RBCs,
- the estimated volume of distribution, or V_d, for the daily dose of 10 mg/kg was 0.7 L/kg, and
- at a dose of 10 mg/kg, the mean plasma concentration of atrazine or its metabolites at steady-state was 104.6 mg-equivalents ^{14}C-label/L of cells.

The RBC:plasma concentration ratio was roughly related linearly in all dose levels. The estimated half-life of 8.14 days and the large volume of distribution 104.6 mg-equivalents/L) in RBCs indicate that extensive binding of atrazine and its metabolites in RBCs was occurring. (The life span of a rat RBC is 45-56 days). The author speculates that binding of ^{14}C-label is of a covalent nature.

Table 3

Red Blood Cell Levels of ^{14}C-Label (ppm) During the Dosing Period and at Sacrifice
(taken from Table IX)

Dose	1 mg/kg		3 mg/kg		7 mg/kg		10 mg/kg		50 mg/kg		100 mg/kg	
Rat #:	R5062	R5063	R5064	R5065	R5066	R5067	R5068	R5069	R5070	R5071	R5072	R5073
Hour of Sacrifice:	3	72	72	3	3	72	3	72	3	72	3	72
Time (hrs.):												
24	0.93	0.54	2.57	4.67	5.19	6.39	7.31	7.81	50.87	36.59	109.06	70.38
48	1.48	1.27	4.29	8.48	12.82	14.17	20.72	15.88	124.35	87.00	234.05	190.48
72	2.64	2.77	--	--	19.98	23.08	31.55	26.71	177.56	129.41	292.78	275.59
96	3.51	2.76	21.47	22.01	26.65	30.38	39.07	37.59	225.49	160.46	474.56	305.37
144	5.18	4.24	--	--	37.07	30.78	60.65	53.79	358.75	289.12	881.41	649.45
192	6.61	5.04	23.44	11.63	43.78	50.05	63.84	63.85	415.80	362.30	695.14	529.04
219	10.03	--	--	21.20	54.98	--	83.92	--	307.74	--	517.23	--
240	--	5.98	18.57	--	--	51.45	--	85.63	--	324.18	--	551.40
264	--	5.41	18.11	--	--	46.65	--	67.83	--	318.95	--	605.28
288	--	5.34	18.50	--	--	50.77	--	41.26	--	271.48	--	611.42

Tissue concentrations of atrazine. The tissue concentrations of atrazine and its metabolites were measured in selected tissues from animals killed at 3 and at 72 hours (Table 4). At all doses, tissue levels of ^{14}C-label are consistently lower in all animals killed 72 hours after cessation of ^{14}C-atrazine exposure, a finding that corroborates the observed decline in plasma concentration of ^{14}C-label (Table 2). The liver had the highest tissue concentration of ^{14}C-label, followed by the kidney, pituitary, ovary and brain. The pectoral and inguinal mammary glands had the lowest tissue concentration in this experiment. In respect to making dose comparisons, tissue levels of ^{14}C-label were generally superlinear, i.e., the tissue level in rats given 100 mg/kg ^{14}C-atrazine was generally 200 times higher than that of rats given 1 mg/kg ^{14}C-atrazine. In animals sacrificed at 72 hours, the mammary tissue:plasma concentration ratio at 1 mg/kg was 0.042 and at 100 mg/kg was 0.49; a difference that is roughly proportional to the dose of atrazine.